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Ordering Information

Mailing Address: Spherotech, Inc.
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Lake Forest, IL 60045
Telephone: 800-368-0822 (Within U.S.A.);
847-680-8922
Fax: 847-680-8927
E-mail: service@spherotech.com
Internet: http://www.spherotech.com

Terms and Conditions

Terms

All payments are due net 30 days, FOB Lake Forest, Illinois. A late payment charge of 1.5% monthly will be added if payment is not received by due date. All purchases are subject to credit approval. Prices and product specification are subject to change without notice.

Payment Options

1. Pay with a check in US$ drawn on a bank in the USA.
2. Charge to VISA or MasterCard

(Please provide the card number, name on the card and expiration date at time of purchase.)

3. Wire transfer to our bank account at PNC Bank, 325 North Milwaukee Ave. Libertyville, IL 60048
Account number: **will be furnished upon request.**
ABA routing number: **041000124**
SWIFT Code: **PNCCUS33 (International).**

Customers with poor payment history are required to use a credit card for purchases.

Shipping

Shipments are freight prepaid via Spherotech preferred carrier. Shipping charges and insurance are prepaid by Spherotech and are added to customer invoice. Claims for lost or damaged products are the responsibility of the purchaser and should be reported to the appropriate carrier within five working days. All lost and damaged goods claims must be confirmed in writing.

Returns

Contact Spherotech for authorization prior to returning material. Spherotech must authorize all returns.

WARRANTY AND LIMITATION OF REMEDY

Spherotech, Inc. makes no warranty of any kind, expressed or implied, including any warranty of fitness for any particular purpose, except that the products sold by Spherotech shall meet its specifications on delivery. Buyer's exclusive remedy and Spherotech's sole liability hereunder shall be limited to, at Spherotech's option, refund of purchase price or the replacement of all material(s) that does not meet its specifications. By acceptance of the product, Buyer indemnifies and holds Spherotech harmless against, and assumes all liability for the consequence of its use or misuse by the Buyer, its employees or others, including, but not limited to, the costs of handling. Said refund or replacement is conditioned on Buyer notifying Spherotech within thirty (30) days of the receipt of product. Failure of Buyer to give said notice within said thirty (30) days shall constitute a waiver by the Buyer of all claims hereunder with respect to said material(s). A 20% restocking fee will be incurred for any returned item, not exchanged or credited due to defect.

Custom Orders

Inquiries for custom prepared particles or labeled particles should be directed to Spherotech technical services.

Reservation of Title

All products remain the property of Spherotech, Inc. until Spherotech, Inc receives full payment.

All products are for Research Use Only (RUO) and are not intended for use in humans or for In-Vitro Diagnostic (IVD) use. No license for any use is expressed or implied.

About Us

Spherotech was established in 1992 to manufacture and supply uniform microparticles for biomedical and diagnostic applications , as well as flow cytometry applications. Our headquarters are 25 miles north of Chicago in Lake Forest, IL.

As a global supplier of microparticle solutions, our experienced scientists manufacture high quality latex, fluorescent, paramagnetic, ferromagnetic and colored dyed microparticles. Our microsphere portfolio offers you:

- Particles with surface chemistry allowing for research analysis using a broad range of crosslinking techniques
  - Microbeads coated with antibodies, proteins, orligands
  - Microbeads functionalized with carboxyl and amino groups
- Particles manufactured with sizes specific to research needs, and crosslinked particles for use with applications using organic solvents
- Flow cytometry grade microbeads for alignment, calibration, compensation, counting, and sorting.

World class quality and technical support are standard practice. As an ISO 9001:2008 registered company, we consistently meet or exceed customer expectations. Spherotech's scientists work to master their knowledge of the world of biotechnological research.

Microparticles manufactured by Spherotech are utilized in:

- Fluorescence immunoassay
- Enzyme immunoassay (EIA)
- Fluorescence microscopy
- Confocal fluorescence microscopy
- Flow cytometry/ image cytometry
- Magnetic cell separation
- Magnetic particles EIA
- Microfluidics
- Other research and industrial applications.

Spherotech specializes in microparticle research and development to introduce new products and add to our ever-expanding portfolio. We constantly introduce new products and improve the existing portfolio. Our loyal customers value Spherotech's agile manufacturing capabilities, custom OEM particle solutions, and value-add supply options. Our manufacturing facilities can accommodate multi-liter lot sizes of our entire microsphere offering.

While we publish an overview of our products, it is by no means comprehensive. If the particle you require is not listed, we urge you to contact our team to check our complete existing or potential inventory.

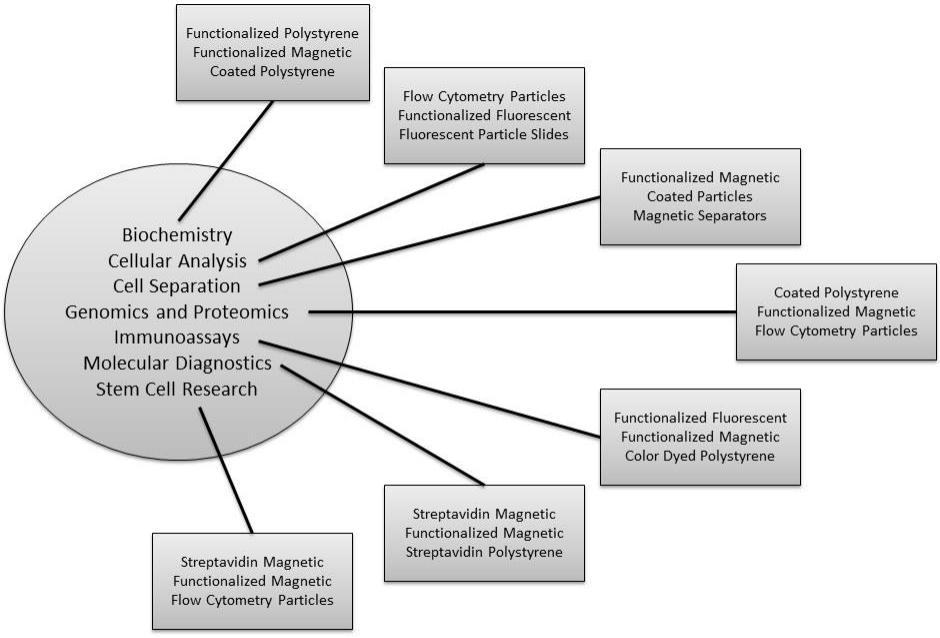


Figure 1 Examples of SPHERO™ Microparticles and their applications.

Product Information

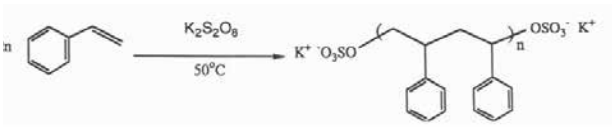
SPHERO™ Polystyrene Particles

- Uniform Shape and Size
- Multi-liter Capabilities
- Available from 0.05 to 200 µm.

The SPHERO™ polystyrene particles are prepared by conventional emulsion polymerization with styrene as the monomer and potassium persulfate or benzoyl peroxide as polymerization initiator. In general, microparticles less than 0.5 µm are prepared in one step. Larger particles are prepared by step wise growing of smaller particles with the addition of styrene monomer and initiator without any additional detergent. The microparticles are cleaned by repeated centrifugation. Cleaned microparticles are resuspended in deionized water. Sodium azide (0.02%) is added as a bacteriostatic. As a result, the SPHERO™ microparticles can be coated with proteins without further cleaning.

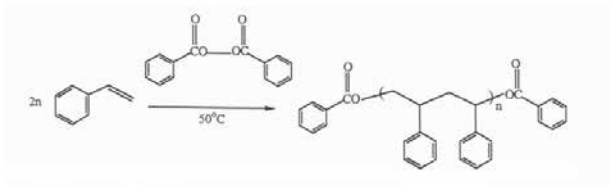
Microparticles made using potassium persulfate as initiator have sulfate groups on their surface. As a result, these particles are negatively charged and are hydrophilic, as shown in equation (1).

(1)



The SPHERO™ polystyrene particles greater than 3 µm are usually prepared using benzoyl peroxide as the initiator. These particles are relatively more hydrophobic, as shown in equation (2).

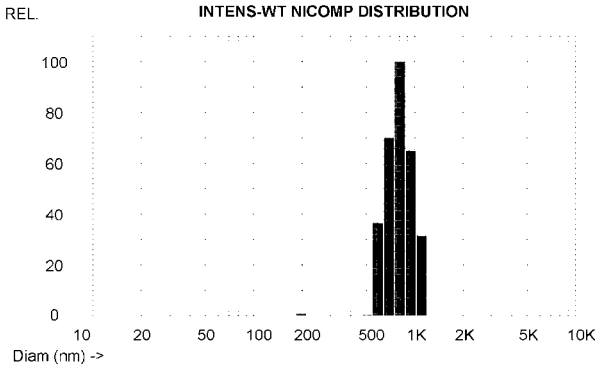
(2)



SPHERO™ polystyrene particles are composed of linear polystyrene without any cross-linking agent. These particles cannot tolerate organic solvents such as toluene, xylene, chloroform, methylene chloride, acetonitrile, dimethyl formamide or acetone. However, SPHERO™ polystyrene particles are stable in the presence of some water miscible solvents such as dimethyl sulfoxide and alcohols. Uniform size cross-linked polystyrene particles that are stable in the presence of organic solvents are also available.

Uniform SPHERO™ polystyrene particles are ideal for use in immunoassays such as latex agglutination, particle base enzyme immunoassays and fluorescence immunoassays. A tight size range of SPHERO™ polystyrene particles is maintained by monitoring size using a NICOMP Laser Particle Sizer (for particles less than 3 µm) and a Scanning Electron Microscope and/or Beckman Coulter Multisizer™ 3 for larger particles. Although the size measurements are accurate, these particles are not certified for use as calibration standard for size measurements or pore size analysis.

Figure 2 Histogram of SPHERO™ 0.8 µm Polystyrene Particles from the NICOMP Laser Particle Sizer.



These polystyrene beads are used during the production of our other product lines including fluorescent, functionalized, crosslinked, magnetic, and protein coated particles. In addition, Spherotech polystyrene beads are used to manufacture a wide range of flow cytometry beads for applications, such as calibration, alignment, sensitivity measurements, compensation, and various kits for assay development.

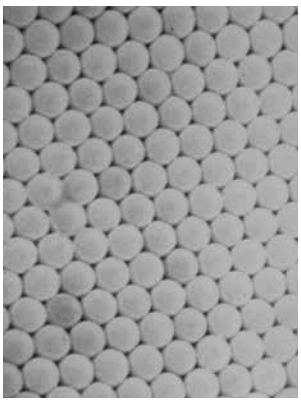
We do not add any additional detergent or surfactant when making our beads to ensure optimal suspensions and functional behavior.

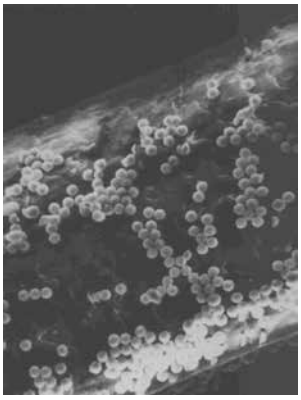
SPHERO™ Polystyrene Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Polystyrene	0.05-0.1	5.0	PP-008-10	10 mL
			PP-008-100	100 mL
Polystyrene	0.1-0.2	5.0	PP-015-10	10 mL
Polystyrene	0.2-0.3	5.0	PP-025-10	10 mL
			PP-025-100	100 mL
Polystyrene	0.4-0.6	5.0	PP-05-10	10 mL
			PP-05-100	100 mL
Polystyrene	0.7-0.9	5.0	PP-08-10	10 mL
			PP-08-100	100 mL
Polystyrene	1.0-1.4	5.0	PP-10-10	10 mL
			PP-10-100	100 mL
Polystyrene	1.5-1.9	5.0	PP-15-10	10 mL
			PP-15-100	100 mL
Polystyrene	2.0-2.4	5.0	PP-20-10	10 mL
			PP-20-100	100 mL
Polystyrene	2.5-2.9	5.0	PP-25-10	10 mL
			PP-25-100	100 mL
Polystyrene	3.0-3.4	5.0	PP-30-10	10 mL
			PP-30-100	100 mL
Polystyrene	3.5-3.9	5.0	PP-35-10	10 mL
			PP-35-100	100 mL
Polystyrene	4.0-4.4	5.0	PP-40-10	10 mL
			PP-40-100	100 mL
Polystyrene	4.5-4.9	5.0	PP-45-10	10 mL
			PP-45-100	100 mL
Polystyrene	5.0-5.9	5.0	PP-50-10	10 mL
			PP-50-100	100 mL
Polystyrene	6.0-8.0	5.0	PP-60-10	10 mL
			PP-60-100	100 mL
Polystyrene	8.0-12.9	2.5	PP-100-10	10 mL

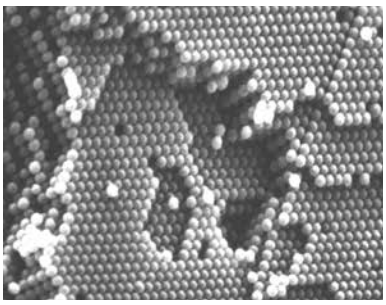
Figure 3 Scanning Electron Microscope (SEM) photos of polystyrene particles are shown below to illustrate the uniformity of their size. (a) Single sheet of 0.8 µm polystyrene particles. (b) 3.4 µm polystyrene particles on the surface of a human hair, which is about 100 µm in diameter. (c) Face-centered-cubic packing of 0.86 µm particles. Theoretically, particles fill ~74% of the space regardless of their size.

(A) **(B)**





(C)

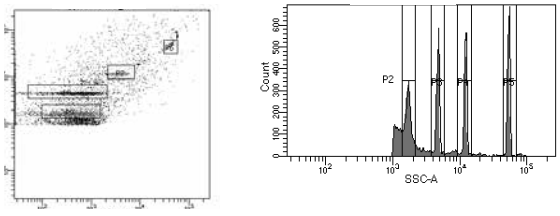


SPHERO™ Nano Polystyrene Particle Size Standard Kit

- Consists of ready to use blank beads with 4 different diameters from ~200 nm to ~1.5 µm
- Designed to estimate the size of microparticles (MPs, 0.5-0.9µm), aquatic bacteria (0.2-0.6µm), and platelets (0.9-3µm) with analytical sizing instrumentation
- Provides a cost effective submicron size standardization substitute for validating instruments when NIST beads are not necessary.

Particle Type and Surface	Catalog No.	Unit
Nano Polystyrene Size Standard Kit, Analytical Grade, 10^6/mL, 0.1-0.3 µm, 0.4-0.6 µm, 0.7-0.9 µm, &1.0-1.9 µm,	NPPS-4K	4x5 mL

Figure 4 Histogram of SPHERO™ Cat. No. NPPS-4K, 0.25, 0.58, 0.79 & 1.34 µm Blank Polystyrene Beads from a BD Bioscience LSRFortessa™ X-20



SPHERO™ Cross-linked Polystyrene Particles

Non-Uniform Cross-linked Particles

- Cost effective alternative if uniform shape is not required
- Uniform size distributions
- Stable in organic solvent.

Spherotech offers a wide range of cross-linked polystyrene particles. Both non-uniform and uniform shaped cross-linked polystyrene particles are manufactured at Spherotech. The low cost non-uniform particles are useful when particle shape does not matter. These non-uniform cross-linked polystyrene particles are stable in the presence of organic solvents. Figure 5 shows the differences between the polymeric particles consisting of polystyrene and particles made from copolymers, styrene/divinylbenzene.

Uniform Cross-linked Particles

- Highly uniform and monodispersed
- Available from 3 to 30 micron
- Stable in only aqueous solvent.

If highly spherical monosized polymer particles are needed, Spherotech also has cross-linked polystyrene particles that are uniform in size and shape. Figures 6 and 7 show Beckman Coulter Multisizer™ 3 histograms for Cat. No. PPX-150-10 (Polystyrene Particles, Cross-linked, 2.5% w/v, 15.2 µm, 10 mL) and Cat. No. CPX-30-10 (Carboxyl Polystyrene Particles, Cross-linked, 5% w/v, 3.3 µm, 10 mL).

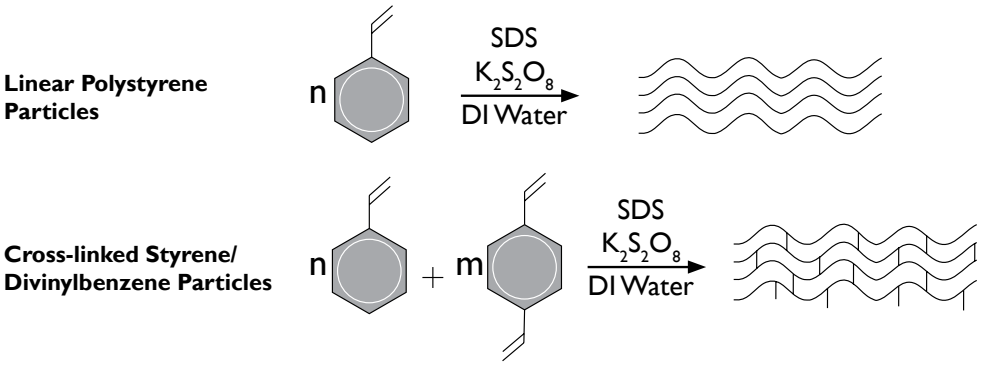
SPHERO™ Non-Uniform Cross-linked Polystyrene Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Polystyrene, Cross-linked, non-uniform shape	0.4-0.6	5.0	PPX-05-10	10 mL
Polystyrene, Cross-linked, non-uniform shape	0.7-0.9	5.0	PPX-08-10	100 mL
Polystyrene, Cross-linked, non-uniform shape	1.0-1.9	5.0	PPX-10-10	10 mL
Polystyrene, Cross-linked, non-uniform shape	2.0-2.4	5.0	PPX-20-10	10 mL
Polystyrene, Cross-linked, non-uniform shape	2.5-2.9	5.0	PPX-25-10	10 mL

SPHERO™ Uniform Cross-linked Polystyrene Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Polystyrene, Cross-linked	5.0-5.9	5.0	PPX-50-10	10 mL
Polystyrene, Cross-linked	8.0-12.9	2.5	PPX-100-10	10 mL
Polystyrene, Cross-linked	13.0-17.9	2.5	PPX-150-10	10 mL
Polystyrene, Cross-linked	18.0-24.9	2.5	PPX-200-10	10 mL
Polystyrene, Cross-linked	25.0-37.0	2.5	PPX-250-10	10 mL

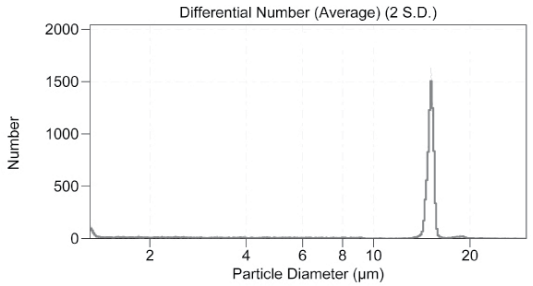
Figure 5 Comparison of linear polystyrene particles and cross-linked copolymers particles made of styrene/divinylbenzene.



SPHERO™ Large Research Grade Cross-linked Polystyrene Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Polystyrene, Cross-linked,	38.0-52.0	5.0	PPX-400-10	10 mL
Polystyrene, Cross-linked,	53.0-69.0	5.0	PPX-600-10	10 mL
Polystyrene, Cross-linked	70.0-89.0	5.0	PPX-800-10	10 mL
Polystyrene, Cross-linked	90.0-105.0	5.0	PPX-1000-10	10 mL
Polystyrene, Cross-linked	106.0-124.0	5.0	PPX-1200-10	10 mL
Polystyrene, Cross-linked	125.0-149.0	5.0	PPX-1400-10	10 mL
Polystyrene, Cross-linked	150.0-175.0	5.0	PPX-1600-10	10 mL
Polystyrene, Cross-linked	176.0-195.0	5.0	PPX-1800-10	10 mL
Polystyrene, Cross-linked	196.0-211.0	5.0	PPX-2000-10	10 mL
Polystyrene, Cross-linked	212.0-249.0	5.0	PPX-2200-10	10 mL

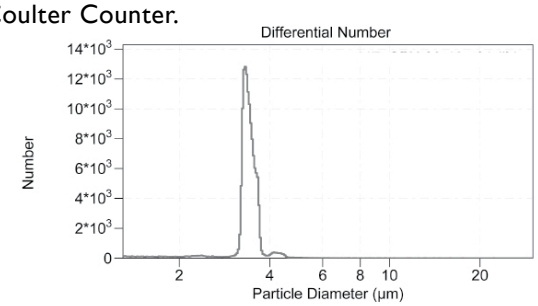
Figure 6 Histogram of SPHERO™ Cat. No. PPX-150-10, 15.2 µm Polystyrene Cross-linked Particles from a Beckman Coulter Multisizer™ 3 Coulter Counter.



SPHERO™ Functionalized Cross-linked Polystyrene Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Carboxyl-polystyrene, Cross-linked	3.0-3.4	5.0	CPX-30-10	10 mL
Carboxyl-polystyrene, Cross-linked	5.0-5.9	2.5	CPX-50-10	10 mL
Carboxyl-polystyrene, Cross-linked	6.0-6.9	2.5	CPX-60-10	10 mL
Carboxyl-polystyrene, Cross-linked	8.0-12.9	2.5	CPX-100-10	10 mL
Carboxyl-polystyrene, Cross-linked	13.0-17.9	2.5	CPX-150-10	10 mL
Carboxyl-polystyrene, Cross-linked	18.0-24.9	2.5	CPX-200-10	10 mL
Amino-polystyrene, Cross-linked	2.0-2.9	1.0	APX-20-10	10 mL
Amino-polystyrene, Cross-linked	3.0-3.4	2.5	APX-30-10	10 mL
Amino-polystyrene, Cross-linked	6.0-6.9	2.5	APX-60-10	10 mL
Amino-polystyrene, Cross-linked	8.0-12.9	2.5	APX-100-10	10 mL

Figure 7 Histogram of SPHERO™ Cat. No. CPX-30-10, 3.3 µm Carboxyl-Polystyrene Cross-linked Particles from a Beckman Coulter Multisizer™ 3 Coulter Counter.

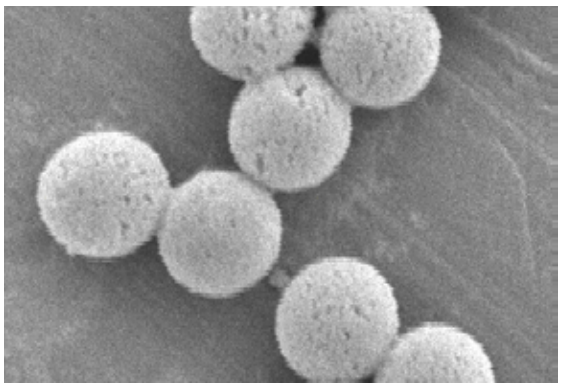


SPHERO™ Porous Cross-linked Polystyrene Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Porous Polystyrene, Cross-linked	8.0-12.9	1.0	PPRXS-100-10	10 mL
Porous Polystyrene, Cross-linked	5.0-5.9	1.0	PPRXS-50-10	10 mL

- Highly uniform and monodispersed
- Consists of 300 to 600 Angstrom pore size
- Exhibits increased surface area over smooth polystyrene beads
- Stable in only aqueous solvents

Figure 8 Scanning Electron Microscope (SEM) photos of 5.1µm porous polystyrene particles with 500 Angstrom pore size.



SPHERO™ Functionalized Polystyrene Particles

Specific particle surface chemistry enables a broad range of coating and binding applications. SPHERO™ functionalized polystyrene particles provide reactive groups on uniform microparticles for consistent and repeatable coating and binding. There are several ways to prepare particles with functionalized surfaces.

- Polymerization initiator selection
- Functionalized monomer grafting

A variety of functional groups can be provided on the microparticle's exterior surface by selecting the appropriate polymerization initiator. For instance, if potassium persulfate is used as an initiator for polymerization the particle will have sulfate groups. Similarly, other functional groups can be introduced on the surface of the particles by using other functionalized initiators.

Another method for providing surface functional groups is by grafting functionalized monomer after the polymerization process. This type of functionalized polystyrene particles are prepared by coating a thin layer of functionalized monomer onto the surface of plain particles. As a result, all of the functional groups are on the surface of the particles. The functional groups are attached to the surface of the particles by alkyl chains of two to eight carbons in length depending upon the type of functionalized monomer used.

Below are the surface charge densities for 0.8 µm carboxyl and amino particles:

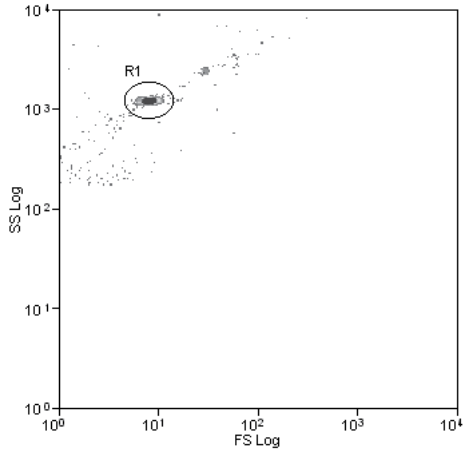
- SPHERO™ carboxyl polystyrene particles with a diameter of 0.8 µm typically contain ~ 50 µeq/g of carboxyl groups on their surface.
- SPHERO™ amino polystyrene particles with a diameter of 0.8 µm typically contain ~ 15 to 20 µeq/g of amino groups on their surface.

Nonetheless, the choice of particle size and type is dependent upon the intended application. For instance, particles with size of 0.4 to 2.0 µm are suitable for latex agglutination assay, solid phase enzyme immunoassay or solid phase fluorescence immunoassay. Particles with size of 2.0 µm or larger are preferred for flow cytometry applications.

SPHERO™ Carboxyl Polystyrene

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Carboxyl-polystyrene	0.05-0.1	2.5	CP-008-20	20 mL
			CP-008-200	200 mL
Carboxyl-polystyrene	0.2-0.3	5.0	CP-025-10	10 mL
			CP-025-100	100 mL
Carboxyl-polystyrene	0.4-0.6	5.0	CP-05-10	10 mL
			CP-05-100	100 mL
Carboxyl-polystyrene	0.7-0.9	5.0	CP-08-10	10 mL
			CP-08-100	100 mL
Carboxyl-polystyrene	1.0-1.4	5.0	CP-10-10	10 mL
			CP-10-100	100 mL
Carboxyl-polystyrene	1.5-1.9	5.0	CP-15-10	10 mL
			CP-15-100	100 mL
Carboxyl-polystyrene	2.0-2.4	5.0	CP-20-10	10 mL
			CP-20-100	100 mL
Carboxyl-polystyrene	2.5-2.9	5.0	CP-25-10	10 mL
			CP-25-100	100 mL
Carboxyl-polystyrene	3.0-3.4	5.0	CP-30-10	10 mL
			CP-30-100	100 mL
Carboxyl-polystyrene	3.5-3.9	5.0	CP-35-10	10 mL
			CP-35-100	100 mL
Carboxyl-polystyrene	4.0-4.4	5.0	CP-40-10	10 mL
			CP-40-100	100 mL
Carboxyl-polystyrene	4.5-4.9	5.0	CP-45-10	10 mL
			CP-45-100	100 mL
Carboxyl-polystyrene	5.0-5.9	5.0	CP-50-10	10 mL
			CP-50-100	100 mL
Carboxyl-polystyrene	6.0-8.0	5.0	CP-60-10	10 mL
			CP-60-100	100 mL

Figure 9 Flow cytometry forward scatter vs. side scatter dot plot for Cat. No. CP-08-10 Lot AD01.



SPHERO™ Amino Polystyrene

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Amino-polystyrene	0.2-0.3	2.5	AP-025-10	10 mL
			AP-025-100	100 mL
Amino-polystyrene	0.4-0.6	5.0	AP-05-10	10 mL
			AP-05-100	100 mL
Amino-polystyrene	0.7-0.9	5.0	AP-08-10	10 mL
			AP-08-100	100 mL
Amino-polystyrene	1.0-1.4	5.0	AP-10-10	10 mL
			AP-10-100	100 mL
Amino-polystyrene	2.0-2.4	5.0	AP-20-10	10 mL
			AP-20-100	100 mL
Amino-polystyrene	2.5-2.9	5.0	AP-25-10	10 mL
			AP-25-100	100 mL
Amino-polystyrene	3.0-3.4	5.0	AP-30-10	10 mL
			AP-30-100	100 mL
Amino-polystyrene	3.5-3.9	5.0	AP-35-10	10 mL
			AP-35-100	100 mL
Amino-polystyrene	6.0-8.0	5.0	AP-60-10	10 mL
			AP-60-100	100 mL
Amino-polystyrene	8.0-12.9	1.0	AP-100-10	10 mL

SPHERO™ Jeffamine Polystyrene

Contains a PEG-based spacer arm that is terminated with amine groups

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Jeffamine®	1.0-1.4	1.0	JAP-10-5	5 mL

JEFFAMINE® is a registered trademark of Huntsman Corporation

SPHERO™ Sulfonate Polystyrene

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Sulfonate-polystyrene	0.7-0.9	5.0	SP-08-10	10 mL

SPHERO™ Hydroxy Polystyrene

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Hydroxy-polystyrene	0.7-0.9	5.0	HP-08-10	10 mL
			HP-08-100	100 mL

SPHERO™ Dimethylamino Polystyrene

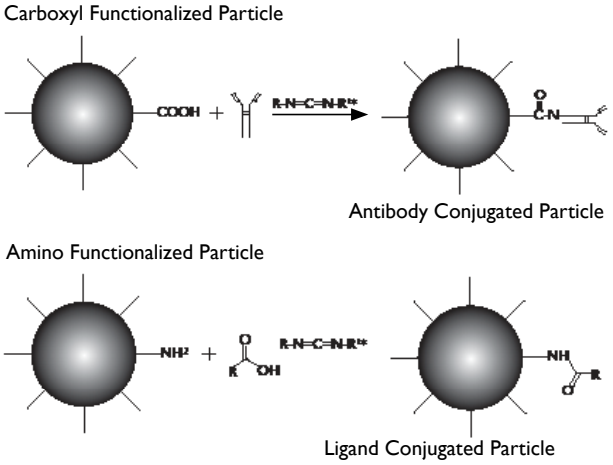
Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Dimethylamino-polystyrnene	0.7-0.9	5.0	DP-08-10	10 mL
			DP-08-100	100 mL

SPHERO™ Functionalized Particle Application Examples and Recommendations:

- Carboxyl or Amino functionalized particles are very useful for covalent coupling of proteins, ligands, antibodies or antigens to the surface of the microparticles using water soluble carbodiimide as the coupling agent. Figure 9 is a diagram of antibody and protein coating of carboxyl and amino particles using EDC coupling.
- Varying particle surface charges can be obtained using functionalized particles such as hydroxyl, sulfate and dimethylamino. These particles are used to manipulate the orientation of the coated material by passive adsorption.
- Polyclonal antibodies can be coated to polystyrene particles by passive adsorption. According to our experience, the optimal amount of antibody to particles ratio is ~100 µg of antibody per mL of 0.5% w/v (5 mg solid per mL) of 0.8 µm polystyrene. Since the total surface area of the particles is inversely proportional to the diameter of the particles, the amount of antibody to particles ratio needs to be adjusted accordingly.
- The washing of polystyrene particles to remove unbound proteins or ligands during coating is accomplished by centrifugation or tangential flow filtration for particles with size of 0.4 µm or larger. For smaller size particles gel filtration, dialysis, or tangential flow filtration should be used.

Please refer to SPHERO™ Recommended Coating Procedures catalog pages for more information.

Figure 10 Examples of carbodiimide-mediated coupling processes.



SPHERO™ Copolymeric Micoparticles

- Uniform Shape and Size
- Multi-liter Capabilities
- Provide more hydrophilic charateristics than styrene-based particles

Spherotech offers microparticles manufactured by copolymerizing styrene with PMMA (polymethylmethacrylate). MMA is an uncharged polar monomer. When copolymerized with styrene the result are beads that are less hydrophobic than pure polystyrene beads. The polystyrene/PMMA copolymer beads are reported to bind less protein than polystyrene; however, the bound protein tends to have better retention of activity. In addition, the polystyrene/PMMA is reported to have less nonspecific binding than polystyrene.

Polymeric beads containing hydroxyl groups have been manufactured by Spherotech. These are created from copolymers of pHEMA (Hydroxyethylmethacrylate) and polystyrene onto a polystyrene core bead. The result are beads that have hydrophilic surface characteristics. In addition, these beads can be activated for covalent coupling. For example, cyanogen bromide, carbonyldiimidazole, and disuccinimidyl carbonate can be used to activate hydroxyl particles to couple to amine-containing ligands.

SPHERO™ PMMA/Polystyrene Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
PMMA/Polystyrene,	1.0-1.4	5.0	PMMP-10-10	10 mL

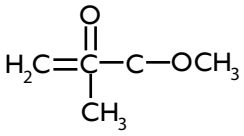
SPHERO™ HEMA/Polystyrene Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
HEMA/Polystyrene,	1.0-1.4	5.0	HEMAP-10-10	10 mL

Figure 11 Structure and properties of PMMA and HEMA Monomers

A. Methylmethacrylate

1. Provides a surface less hydrophobic than polystyrene
2. Exhibits less autofluorescence than polystyrene beads.



B. Hydroxyethylmethacrylate

1. Provides a surface with hydroxyl groups
2. Can be activated for coupling amine-containing ligands.

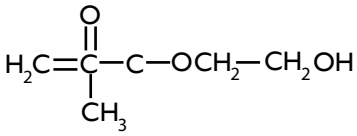


Figure 12 Histogram of SPHERO™ Cat. No. PMMP-10-10, 1.4 µm PMMA/Polystyrene Copolymeric Particles from a Beckman Coulter Multisizer™ 3 Coulter Counter.

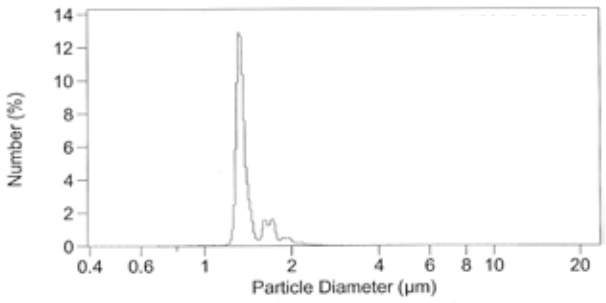
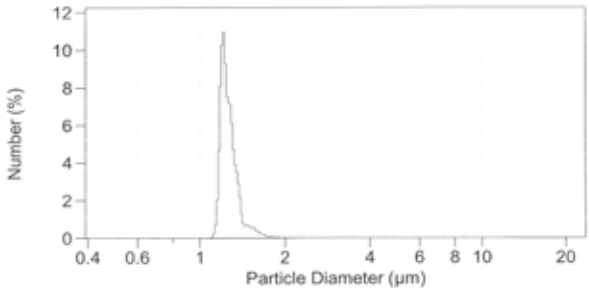


Figure 13 Histogram of SPHERO™ Cat. No. HEMAP-10-10, 1.3 µm HEMA/Polystyrene Copolymeric Particles from a Beckman Coulter Multisizer™ 3 Coulter Counter.



SPHERO™ Blue Particles

- Excellent for latex agglutination tests
- Enhances the visibility of agglutination
- Available with functional groups for covalent binding of antigens or antibodies.

The SPHERO™ Blue Particles are prepared by polymerizing oil-soluble dye in styrene. They are free of solvent commonly found in particles stained with a solution of dye in organic solvent. These particles are intensely colored to enhance the visual detection in assays such as latex agglutination (Figures 15 & 16), dipstick and membrane based assays.

SPHERO™ Polystyrene Blue Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Blue Polystyrene	0.4-0.6	5.0	PPB-05-10	10 mL
Blue Polystyrene	0.4-0.6	5.0	PPB-05-100	100 mL
Blue Polystyrene	6.0-8.0	1.0	PPB-60-5	5 mL
Blue Polystyrene	90-105	1.0	PPB-1000-5	5 mL

SPHERO™ Carboxyl Blue Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Carboxyl Blue	0.03-0.06	5.0	CPB-005-10	10 mL
Carboxyl Blue	0.1-0.19	5.0	CPB-01-10	10 mL
Carboxyl Blue	0.1-0.19	5.0	CPB-01-100	100 mL
Carboxyl Blue	0.2-0.29	5.0	CPB-02-10	10 mL
Carboxyl Blue	0.2-0.29	5.0	CPB-02-100	100 mL
Carboxyl Blue	0.3-0.39	5.0	CPB-03-10	10 mL
Carboxyl Blue	0.3-0.39	5.0	CPB-03-100	100 mL
Carboxyl Blue	0.4-0.6	5.0	CPB-05-10	10 mL
Carboxyl Blue	0.4-0.6	5.0	CPB-05-100	100 mL

Figure 14 SEM photo of Cat. No. CPB-05-10, 0.41 µm, 5000X.

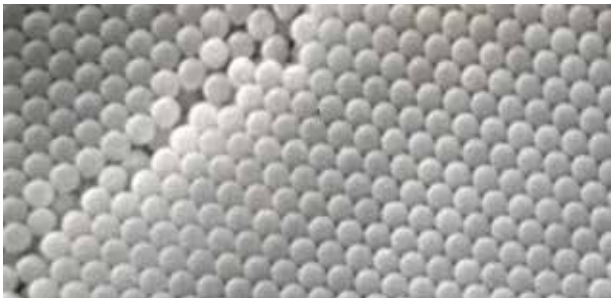


Figure 15 Two forms of simple latex agglutination: (1) The antibody is coated to the particles and reacts with the antigen in the test sample. (2) The antigen is coated to the particles and reacts with the antibody in the test sample.

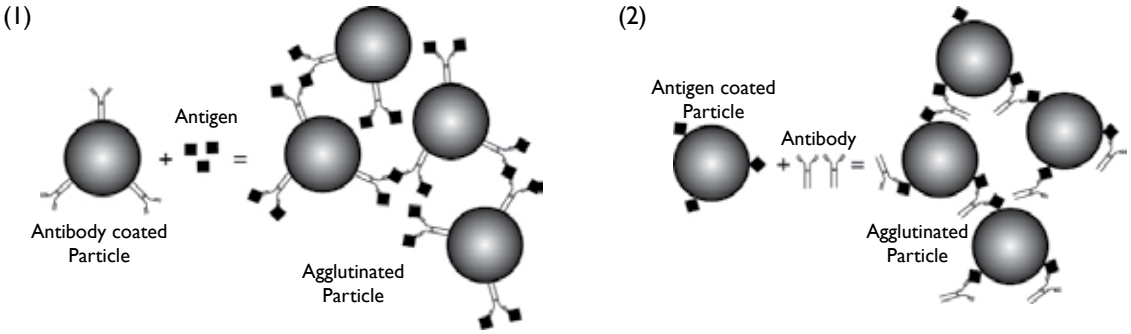
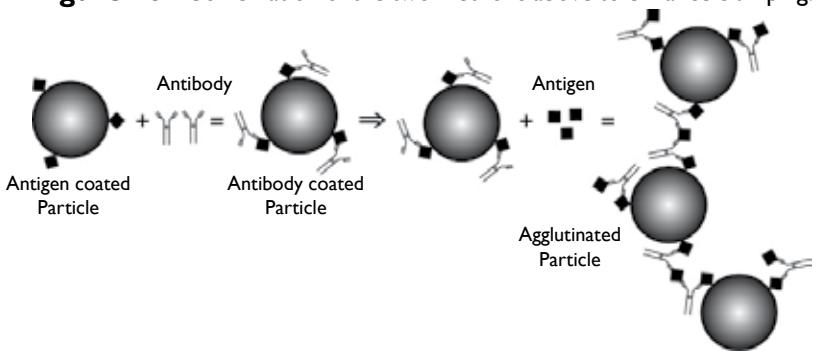


Figure 16 Combination of the two methods above to enhance clumping.

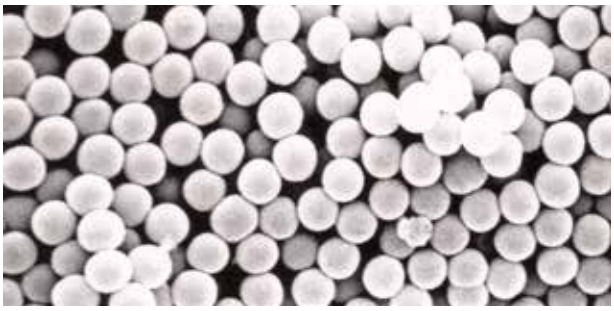


SPHERO™ Silica Particles

- Excellent mechanical strength
- Low thermal stress
- Very low cation and water content
- Allows excellent light transmission from UV to near IR.

SPHERO™ Silica Particles are non-porous, spherical in shape and very uniform in size as shown in the SEM photo below. The silica particles are heavier than polystyrene particles with a density of 1.96 g/cm^3 and can withstand temperatures of up to 1000 °C. They have been used to adsorb DNA and RNA from cell lysates.

Figure 17 SEM photo of Cat. No. SIP-10-10, 1.23 µm, 1000X.



Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Silica	0.4-0.6	5.0	SIP-05-10	10 mL
Silica	1.0-1.4	5.0	SIP-10-10	10 mL
Silica	1.5-1.9	5.0	SIP-15-10	10 mL
Silica	3.0-3.4	5.0	SIP-30-10	10 mL

Figure 18 Histogram of SPHERO™ Cat. No. SIP-05-10, 0.56 µm Silica Particles from the NICOMP Laser Particle Sizer.

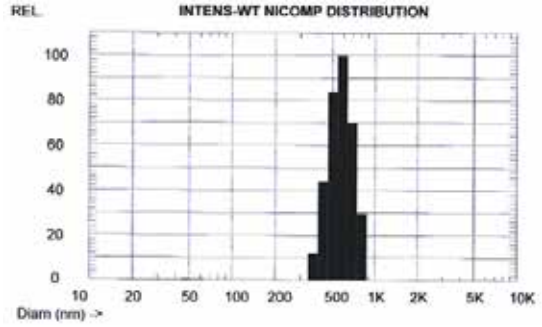
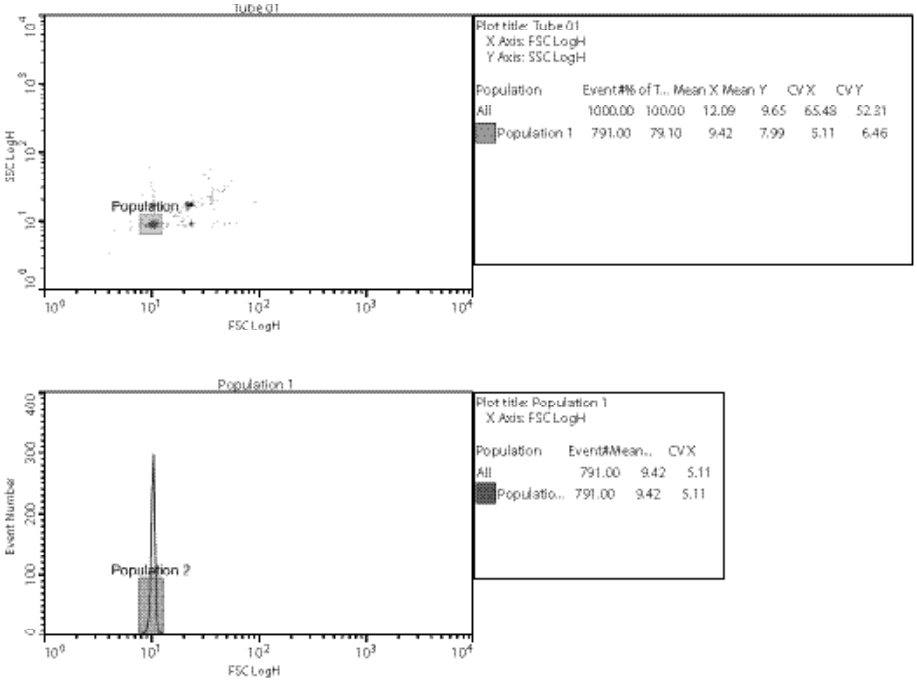


Figure 19 Histograms of Cat. No. SIP-15-10 on a Stratedigm S1400.



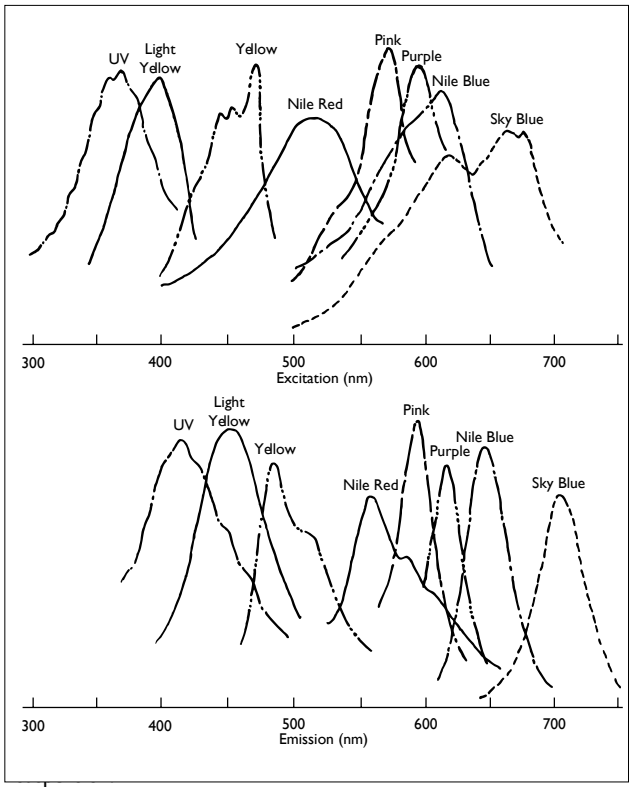
SPHERO™ Fluorescent Particles

- Beneficial to bioimaging and biosensing applications
- Uniform and stable fluorescence
- Available with functional groups for covalent binding

The SPHERO™ fluorescent microparticles are prepared by either staining polystyrene particles with a fluorophore solution or by polymerizing a fluorophore in styrene in the presence of polystyrene core particles. As a result, a wide variety of fluorescent particles can be prepared ranging in size, type of fluorophore, fluorescence intensity and surface functional groups. The fluorophores chosen for use in the preparation of SPHERO™ fluorescent particles are water insoluble and therefore are very stable. These fluorophores, once incorporated into the particles, do not leach and their color and fluorescence remains stable for long periods of time under proper storage conditions.

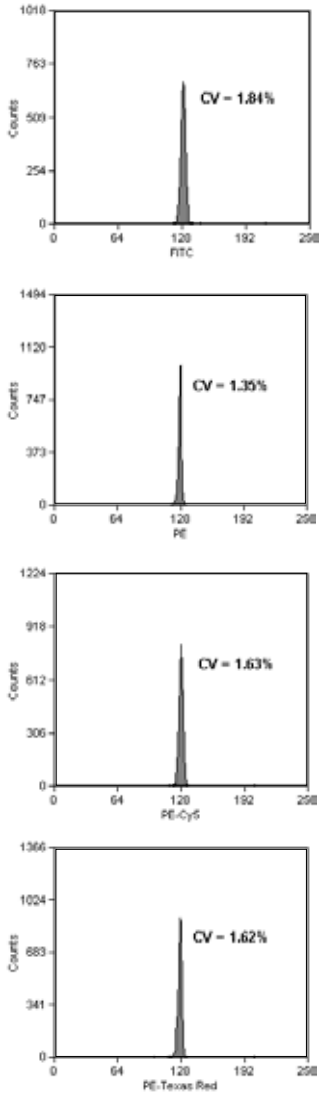
The excitation and emission spectra of some of the fluorophores used in the SPHERO™ fluorescent particles are shown in Figure 19.

Figure 20 Excitation & Emission Spectra of the fluorophores used in SPHERO™ fluorescent particles.



The SPHERO™ fluorescent particles are available in single or multiple fluorophores of various sizes and fluorescence intensities with very small coefficient of variation in both size and fluorescence. They can be used for latex agglutination, fluorescence microscopy, confocal fluorescence microscopy. Many of these particles can be used for flow cytometry. The flow cytometer histograms of 2.9 µm Nile Red Particles (Catalog # FP-3056-2) at four channels are shown in Figure 20. More flow cytometry data for SPHERO™ fluorescent particles is shown on page 15.

Figure 21 Flow cytometry histograms of 2.9 µm, Nile Red Particles (Cat. No. FP-3056-2)



The flow cytometry data of some of the fluorescent particles are shown in **Table I**.

Table I

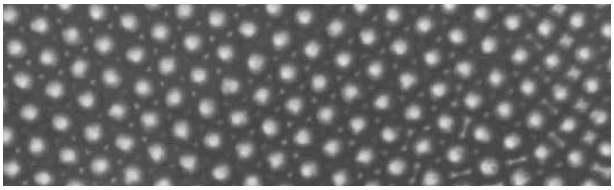
Fluorophores	Cat. No.	Size (µm)	%CV*								
			Violet 1	Violet 2	FL	PE	TR	PE-Cy5	PE-Cy7	APC	APC-Cy7
SPHERO™ Fluorescent Particles, 1.7-2.2 µm											
UV, Low Intensity	FL-2040-2	1.81	6.34	14.51							
UV, High Intensity	FH-2040-2	2.14	3.65								
Light Yellow, Low Intensity	FL-2045-2	2.10	3.89	4.40							
Light Yellow, High Intensity	FH-2045-2	2.16	3.26	3.25							
Yellow, Low Intensity	FL-2052-2	1.80	4.04	4.08	4.53	5.60					
Yellow, High Intensity	FH-2052-2	1.84			2.30	3.50					
Nile Red, Low Intensity	FL-2056-2	2.07		8.88	4.22	3.89	4.34	4.9	9.67		
Nile Red, High Intensity	FH-2056-2	2.27			2.80	2.64	2.55	3.00	5.10		
Pink	FP-2058-2	1.80				2.86	3.55	7.92			
Purple, Low Intensity	FL-2062-2	1.93					10.33				
Purple, Mid-level Intensity	FP-2062-2	2.0					7.13	13.0	10.99		
Purple, High Intensity	FH-2062-2	1.80					5.33	8.02	9.55		
Sky Blue	FP-2070-2	2.07								10.18	11.23
SPHERO™ Fluorescent Particles, 2.5-4.5 µm											
Yellow	FP-4052-2	4.10	3.42	3.47	2.65	2.83	5.15				
Nile Red	FP-3056-2	2.88	3.88	2.70	2.49	2.09	2.10	2.53	5.28		
Nile Blue	FP-3065-2	3.00	8.62	8.10	6.23	6.24	6.59	7.77	13.89	4.99	6.99
Blue	FP-3068-2	3.30								2.78	4.83
SPHERO™ Multiple Fluorophore Particles, 1.7-2.2 µm											
UV/LY	FP-2042-2	2.00									
PR/Y, Low Intensity	FL-2060-2	2.20	2.68	2.66	3.32	3.44	5.02				
PR/Y, Mid-level Intensity	FP-2060-2	2.20	2.88	2.87	2.48	3.35	3.87	6.18			
PR/Y, High Intensity	FH-2060-2	2.02	3.75	3.69	3.50	4.00	2.79	3.86	12.65		
SPHERO™ Multiple Fluorophore Particles, 2.5-5.0 µm											
UV/LY	FP-3042-2	3.20	4.22	4.55							
PK/Y	FP-3055-2	3.00									
PR/Y	FP-4060-2	4.00	3.30	4.05	3.94	4.00	5.56	12.86			
SPHERO™ Fluorescent Particles, 0.7-0.9 µm											
Yellow	FP-0852-2	0.85			5.76	8.14					
Pink	FP-0858-2	0.91				8.22	9.65				
Purple	FP-0862-2	0.84					6.77				

*Data for particles with sizes above 1.0 micron obtained using a Dako Cyan ADP with the following Excitation and Emission wavelength: Violet 1: Ex 405nm, Em. 450/50nm; Violet 2: Ex. 405nm, Em. 530/40nm; FITC: Ex. 488nm, Em. 530/40nm; PE: Ex. 488nm, Em. 575/25nm; TR: Ex 488nm, Em. 613/20nm; PE-Cy5: Ex 488nm, Em. 680/30nm ; PE-Cy7: Ex 488nm, Em. >750nm ; APC: Ex. 633nm, Em. 665/20nm; APC-Cy7: 633nm, Em.> 750 nm

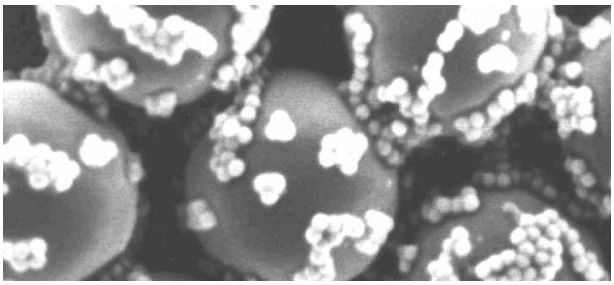
**Data for particles with sizes below 1.0 micron obtained using a Stratedigm S1400 with the following Excitation and Emission wavelength: FITC: Ex. 488nm, Em. 530/30nm; PE: Ex. 488nm, Em. 545/60nm; TR: Ex 488nm, Em. 615/30nm

Figure 22 Microscope photos (a) 10 µm Nile Red beads (40x), (b) 0.4 µm Avidin fluorescent beads binding to the surface of 6.0 µm Biotin polystyrene beads (5000x).

(a)



(b)



SPHERO™ Fluorescent Polystyrene

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Yellow	0.04-0.09	1.0	FP-00552-2	2 mL
Nile Red	0.04-0.06	1.0	FP-00556-2	2 mL
Pink	0.04-0.09	1.0	FP-00558-2	2 mL
Purple	0.04-0.06	1.0	FP-00562-2	2 mL
Sky Blue	0.04-0.09	0.25	FP-00570-2	2 mL
Light Yellow	0.1-0.3	1.0	FP-0245-2	2 mL
Yellow	0.1-0.3	1.0	FP-0252-2	2 mL
Nile Red	0.1-0.3	1.0	FP-0256-2	2 mL
Purple	0.1-0.3	1.0	FP-0262-2	2 mL
Sky Blue	0.1-0.3	0.25	FP-0270-2	2 mL
Light Yellow	0.4-0.6	1.0	FP-0545-2	2 mL
Yellow	0.4-0.6	1.0	FP-0552-2	2 mL
Nile Red	0.4-0.6	1.0	FP-0556-2	2 mL
Pink	0.4-0.6	1.0	FP-0558-2	2 mL
Purple	0.4-0.6	1.0	FP-0562-2	2 mL
Sky Blue	0.4-0.6	1.0	FP-0570-2	2 mL
Light Yellow	0.7-0.9	1.0	FP-0845-2	2 mL
Yellow	0.7-0.9	1.0	FP-0852-2	2 mL
Nile Red	0.7-0.9	1.0	FP-0856-2	2 mL
Pink	0.7-0.9	1.0	FP-0858-2	2 mL
Purple	0.7-0.9	1.0	FP-0862-2	2 mL
Blue	0.7-0.9	1.0	FP-0868-2	2 mL
Sky Blue	0.7-0.9	1.0	FP-0870-2	2 mL
Jade Green	0.7-0.9	1.0	FP-0878-2	2 mL
Yellow	1.0-1.9	1.0	FP-1552-2	2 mL
Light Yellow, Medium Intensity	1.7-2.2	1.0	FP-2045-2	2 mL
Nile Red	1.7-2.2	1.0	FP-2056-2	2 mL
Pink, Medium Intensity,	1.7-2.2	1.0	FP-2058-2	2 mL
Purple , Medium Intensity	1.7-2.4	1.0	FP-2062-2	2 mL
Nile Blue	1.7-2.2	1.0	FP-2065-2	2 mL
Blue	1.7-2.2	1.0	FP-2068-2	2 mL
Sky Blue	1.7-2.2	1.0	FP-2070-2	2 mL
Yellow	2.5-4.5	1.0	FP-3052-2	2 mL
Nile Red	2.5-4.5	1.0	FP-3056-2	2 mL
Pink	2.5-3.4	1.0	FP-3058-2	2 mL
Nile Blue	2.5-4.5	1.0	FP-3065-2	2 mL

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Blue	2.5-4.5	1.0	FP-3068-2	2 mL
Ocean Blue	2.5-4.5	1.0	FP-3069-2	2 mL
Sky Blue	2.5-4.5	1.0	FP-3070-2	2 mL
Yellow	2.5-4.5	1.0	FP-4052-2	2 mL
Nile Red	2.5-4.5	1.0	FP-4056-2	2 mL
Pink	2.5-4.5	1.0	FP-4058-2	2 mL
Purple	2.5-4.5	1.0	FP-4062-2	2 mL
Sky Blue	2.5-4.5	0.2	FP-4070-2	2 mL
Nile Red	5.0-7.9	1.0	FP-5056-10	10 mL
Yellow	5.0-7.9	1.0	FP-6052-2	2 mL
Nile Red	5.0-7.9	1.0	FP-6056-2	2 mL
Pink	6.0-8.0	1.0	FP-6058-2	2 mL
Ocean Blue	5.0-7.9	0.2	FP-6069-2	2 mL
Yellow	7.0-7.9	1.0	FP-7052-2	2 mL
Sky Blue	7.0-7.9	0.25	FP-7070-2	2 mL
UV	10.0-14.0	1.0	FP-10040-2	2 mL
Light Yellow	10.0-14.0	1.0	FP-10045-2	2 mL
Yellow	10.0-14.0	1.0	FP-10052-2	2 mL
Nile Red	10.0-14.0	1.0	FP-10056-10	10 mL
Nile Red	10.0-14.0	1.0	FP-10056-2	2 mL
Purple	10.0-14.0	1.0	FP-10062-2	2 mL
CyBlue	10.0-14.0	10^7/mL	FP-10066-2	2 mL
PAK Blue	10.0-14.0	10^7/mL	FP-10067-2	2 mL
Sky Blue	10.0-14.0	0.2	FP-10070-2	2 mL
UV	15.0-19.0	1.0	FP-15040-2	2 mL
Light Yellow	15.0-19.0	1.0	FP-15045-2	2 mL
Yellow	15.0-19.0	1.0	FP-15052-2	2 mL
Nile Red	15.0-19.0	1.0	FP-15056-2	2 mL
Purple	15.0-19.0	1.0	FP-15062-2	2 mL
CyBlue	15.0-19.0	10^7/mL	FP-15066-2	
PAK Blue	15.0-19.0	1.0	FP-15067-2	2 mL
Sky Blue	15.0-19.0	0.2	FP-15070-2	2 mL
Yellow	18.0-24.9	1.0	FP-20052-5	5 mL
Nile Red	18.0-24.9	1.0	FP-20056-5	5 mL
Yellow	25.0-35.0	1.0	FP-30052-5	5 mL
Nile Red	25.0-35.0	1.0	FP-30056-5	5 mL
Purple	25.0-35.0	1.0	FP-30062-5	5 mL

SPHERO™ Low Intensity Fluorescent Polystyrene

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Light Yellow	0.7-0.9	1.0	FL-0845-2	2 mL
Yellow	0.7-0.9	1.0	FL-0852-2	2 mL
UV	1.7-2.2	1.0	FL-2040-2	2 mL
Light Yellow	1.7-2.2	1.0	FL-2045-2	2 mL
Yellow	1.7-2.2	1.0	FL-2052-2	2 mL
Nile Red	1.7-2.2	1.0	FL-2056-2	2 mL
Purple	1.7-2.2	1.0	FL-2062-2	2 mL
Blue	1.7-2.2	1.0	FL-2068-2	2 mL
Blue	2.5-4.5	1.0	FL-3068-2	2 mL
Sky Blue	3.6-4.5	1.0	FL-4070-2	2 mL
Nile Red	5.0-7.9	1.0	FL-6056-2	2 mL
Blue	6.0-8.0	1.0	FL-6068-2	2 mL
Nile Red	10.0-14.0	1.0	FL-10056-2	2 mL

SPHERO™ Fluorescent PMMA

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Yellow	38.0-44.0	1.0	FPMA-40052-5	5 mL
Nile Red	38.0-44.0	1.0	FPMA-40056-5	5 mL
Purple	38.0-44.0	1.0	FPMA-40062-5	5 mL
Nile Red	45.0-52.0	1.0	FPMA-50056-5	5 mL
Purple	53.0-62.0	1.0	FPMA-60062-5	5 mL

SPHERO™ Multiple Fluorophore Fluorescent Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Multiple Fluorophore	0.1-0.3	0.2	FP-0257-2	2 mL
Multiple Fluorophore	0.4-0.6	0.2	FP-0557-2	2 mL
Multiple Fluorophore	0.7-0.9	0.2	FP-0857-2	2 mL
Multiple Fluorophore	1.7-2.2	0.2	FP-2057-2	2 mL
Multiple Fluorophore	2.5-5.0	0.2	FP-3057-2	2 mL
Multiple Fluorophore	6.0-7.9	1.0	FP-6057-2	2 mL
UV / Light Yellow	0.7-0.9	1.0	FP-0842-2	2 mL
UV / Light Yellow	1.7-2.2	1.0	FP-2042-2	2 mL
UV / Purple / Yellow / Pink / Nile Blue	1.7-2.2	1.0	FP-2054-2	2 mL
Purple / Yellow	1.7-2.2	1.0	FP-2060-2	2 mL
UV / Light Yellow	2.5-5.0	1.0	FP-3042-2	2 mL
Pink / Yellow	2.5-5.0	1.0	FP-3055-2	2 mL
Nile Red / Blue	2.5-4.5	1.0	FP-3066-2	2 mL
Purple / Yellow	2.5-5.0	1.0	FP-4060-2	2 mL
Nile Red / Blue	4.6-5.9	1.0	FP-5066-2	2 mL
Purple / Yellow, High Intensity	1.7-2.2	1.0	FH-2060-2	2 mL
Purple / Yellow, Low Intensity	1.7-2.2	1.0	FL-2060-2	2 mL

SPHERO™ High Intensity Fluorescent Polystyrene

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
UV	1.7-2.2	1.0	FH-2040-2	2 mL
Light Yellow	1.7-2.2	1.0	FH-2045-2	2 mL
Yellow	1.7-2.2	1.0	FH-2052-2	2 mL
Nile Red	1.7-2.2	1.0	FH-2056-2	2 mL
Purple	1.7-2.2	1.0	FH-2062-2	2 mL
Sky Blue	1.7-2.2	0.2	FH-2070-2	2 mL
Nile Red	2.5-4.5	1.0	FH-3056-2	2 mL
Nile Red	5.0-7.9	1.0	FH-5056-2	2 mL
UV	10.0-14.0	1.0	FH-10040-2	2 mL
Yellow	10.0-14.0	1.0	FH-10052-2	2 mL
Nile Red	10.0-14.0	1.0	FH-10056-10	10 mL
Nile Red	10.0-14.0	1.0	FH-10056-2	2 mL
Purple	10.0-14.0	1.0	FH-10062-2	2 mL
Purple	15.0-19.0	1.0	FH-15062-2	2 mL

SPHERO™ FITC Polystyrene Particles

- Surfaced labeled with FITC
- Used as calibration particles for flow cytometry
- Also used to cross calibrate different flow cytometers for data normalization.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
FITC	0.7-0.9	0.1	FICP-08-2	2 mL
FITC	2.0-2.9	0.1	FICP-20-2	2 mL
FITC	3.0-3.9	0.1	FICP-30-2	2 mL
FITC	5.0-5.9	0.1	FICP-50-2	2 mL
FITC	7.0-7.9	0.1	FICP-70-2	2 mL
FITC	8.0-8.9	0.1	FICP-80-2	2 mL

Figure 23 Flow cytometry histograms for Cat. No. FH-2052-2 lot AC01 (Fluorescent Particles, Yellow, High Intensity, 1% w/v, 1.84 µm, 2 mL).

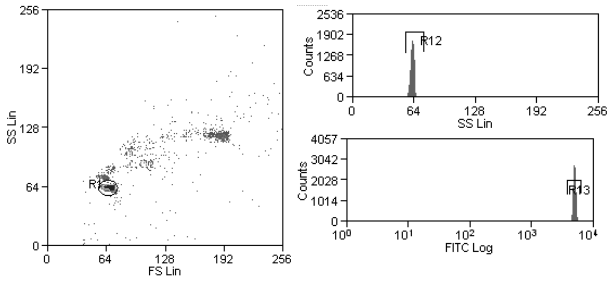


Figure 24 Histogram of SPHERO™ Cat. No. FICP-30-2 (FITC Polystyrene Particles, 0.1% w/v, 3.72 µm , 2 mL) from a BD Bioscience LSRFortessa™ X-20

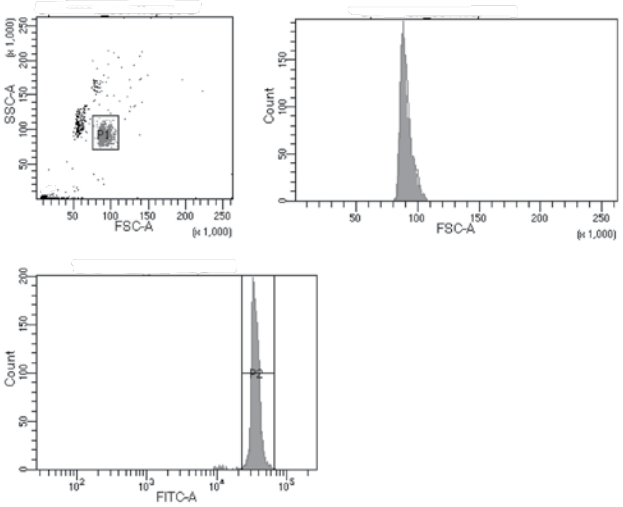


Figure 25 Histogram of SPHERO™ Cat. No. FP-10045-2 (Fluorescent Particles, Light Yellow, 1% w/v, 10.8 µm, 2 mL) from a BD Bioscience LSRFortessa™ X-20

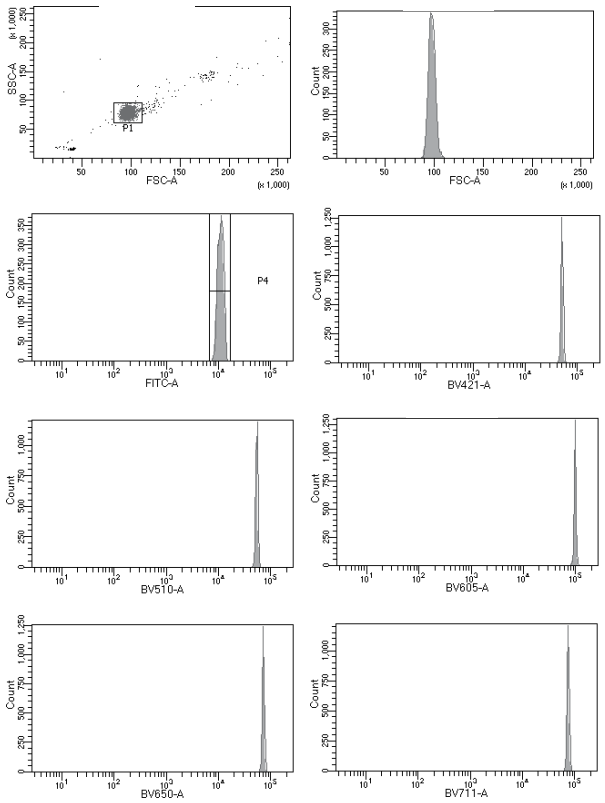


Figure 26 Histogram of SPHERO™ Cat. No. FICP-20-2 (FITC Polystyrene Particles, 0.1% w/v, 1.87 µm , 2 mL) from a BD Bioscience LSRFortessa™ X-20

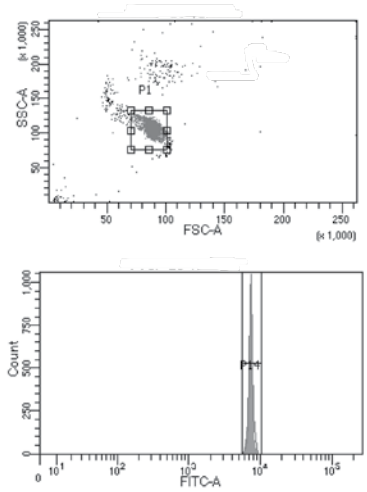
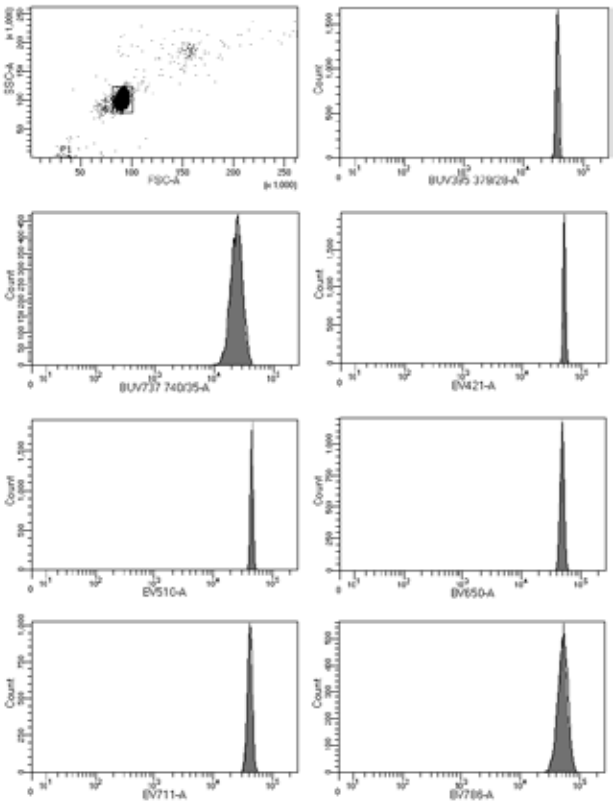


Figure 27 Histogram of SPHERO™ Cat. No. FP-10040-2 (Fluorescent Particles, UV, 1% w/v, 10.2 µm from a BD Bioscience LSRFortessa™ X-20



SPHERO™ Functionalized Fluorescent Particles

- Used for covalent coupling of proteins or ligands
- Can be coated with Avidin, Biotin, Protein A, Goat anti-Mouse IgG, or other protein of interest
- Used as fluorescent tracers for cell surface markers in fluorescence microscopy and flow cytometry.

SPHERO™ Amino Fluorescent Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Yellow	0.09-0.3	1.0	AFP-0252-2	2 mL
Nile Red	0.09-0.3	1.0	AFP-0256-2	2 mL
Yellow	0.4-0.6	1.0	AFP-0552-2	2 mL
Pink	0.4-0.6	1.0	AFP-0558-2	2 mL
Yellow	0.7-0.9	1.0	AFP-0852-2	2 mL
Nile Red	0.7-0.9	1.0	AFP-0856-2	2 mL
Pink	0.7-0.9	1.0	AFP-0858-2	2 mL
Purple	0.7-0.9	1.0	AFP-0862-2	2 mL
Nile Blue	0.7-0.9	1.0	AFP-0865-2	2 mL
Yellow	38.0-44.0	1.0	AFP-40052-5	5 mL

SPHERO™ Carboxyl High Intensity Fluorescent Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Yellow	5.0-5.9	0.5	CFH-5052-2	2 mL
Nile Red	5.0-5.9	0.5	CFH-5056-2	2 mL
Pink	5.0-5.9	0.5	CFH-5058-2	2 mL
Nile Blue	5.0-5.9	0.5	CFH-5065-2	2 mL

SPHERO™ Carboxyl Low Intensity Fluorescent Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Yellow	0.7-0.9	1.0	CFL-0852-2	2 mL
Yellow	5.0-5.9	0.5	CFL-5052-2	2 mL
Pink	5.0-5.9	0.5	CFL-5058-2	2 mL
Nile Blue	5.0-5.9	0.5	CFL-5065-2	2 mL

SPHERO™ Carboxyl Multiple Fluorophore Fluorescent Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
UV / Light Yellow / Yellow	0.04-0.08	1.0	CFP-00546-2	2 mL

SPHERO™ Carboxyl Fluorescent Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Yellow	0.04-0.08	1.0	CFP-00552-2	2 mL
Nile Red	0.04-0.08	1.0	CFP-00556-2	2 mL
Pink	0.04-0.08	1.0	CFP-00558-2	2 mL
Sky Blue	0.04-0.08	0.5	CFP-00570-2	2 mL
Yellow	0.09-0.3	1.0	CFP-0252-2	2 mL
Nile Red	0.09-0.3	1.0	CFP-0256-2	2 mL
Pink	0.09-0.3	1.0	CFP-0258-2	2.2 mL
Purple	0.09-0.3	1.0	CFP-0262-2	2 mL
Yellow	0.4-0.6	1.0	CFP-0552-2	2 mL
Nile Red	0.4-0.6	1.0	CFP-0556-2	2 mL
Pink	0.4-0.6	1.0	CFP-0558-2	2 mL
Purple	0.4-0.6	1.0	CFP-0562-2	2 mL
Sky Blue	0.4-0.6	0.25	CFP-0570-2	2 mL
Yellow	0.7-0.9	1.0	CFP-0852-2	2 mL
Nile Red	0.7-0.9	1.0	CFP-0856-2	2 mL
Pink	0.7-0.9	1.0	CFP-0858-2	2 mL
Purple	0.7-0.9	1.0	CFP-0862-2	2 mL
Sky Blue	0.7-0.9	1.0	CFP-0870-2	2 mL
Yellow	1.7-2.2	1.0	CFP-2052-2	2 mL
Pink	1.7-2.2	1.0	CFP-2058-2	2 mL
Purple	1.7-2.2	1.0	CFP-2062-2	2 mL
CyBlue	1.7-2.2	0.5	CFP-2066-2	2 mL
Sky Blue	1.7-2.2	0.5	CFP-2070-2	2 mL
InfraBlue	1.7-2.2	0.5	CFP-2072-2	2 mL
UV	4.0-4.5	1E8/mL	CFP-4041-2	2 mL
Purple	4.0-4.5	1.0	CFP-4062-2	2 mL
Light Yellow	5.0-5.9	0.5	CFP-5045-2	2 mL
Yellow	5.0-5.9	0.5	CFP-5052-2	2 mL
Nile Red	5.0-5.9	0.5	CFP-5056-2	2 mL
Pink	5.0-5.9	0.5	CFP-5058-2	2 mL
Nile Blue	5.0-5.9	0.5	CFP-5065-2	2 mL
CyBlue	5.0-5.9	1.0	CFP-5066-2	2 mL
Sky Blue	5.0-5.9	0.5	CFP-5070-2	2 mL
Nle Red	10.0-14.0	0.5	CFP-10056-2	2 mL
Nile Red	15.0-19.0	0.5	CFP-15056-2	2 mL

SPHERO™ Dimethylamino Fluorescent Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Purple	2.5-3.5	1.0	DFP-3062-2	2 mL

SPHERO™ Calibration Particles

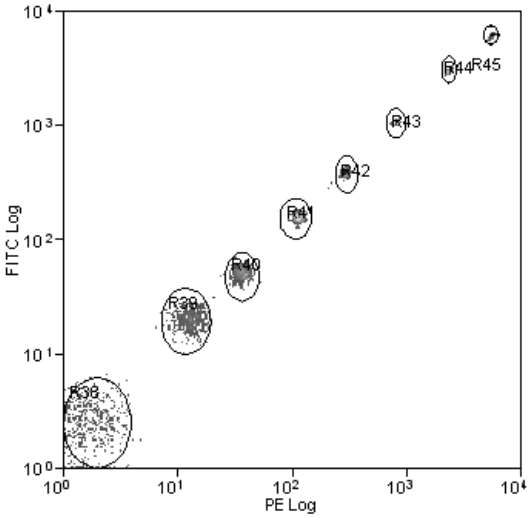
SPHERO™ Calibration Particles are designed for routine calibration of flow cytometers. They are used extensively by many laboratories for QC and long term performance tracking of the flow cytometer. In addition, they are also used for routine alignment and calibration in fluorescence and confocal fluorescence microscopy.

SPHERO™ Rainbow Calibration Particles

- Contains multiple fluorophores incorporated in the same particle to be used in multiple channels of the flow cytometer
- Available with different fluorescent intensities on the same size particles
- Stable for several years when stored properly
- Withstand freeze-thaw cycles; diluted particles can be stored frozen for later use
- Can be sanitized by treating with 70% ethanol or other antibiotic agents.

Particle Type and Surface	Size, µm	Catalog No.	Unit
Rainbow Calibration, 4 peaks, 10^7/mL	1.8-2.2	RCP-20-5	5 mL
Rainbow Calibration, 6 peaks, 10^7/mL	3.0-3.4	RCP-30-5	5 mL
Rainbow Calibration, 8 peaks, 10^7/mL	3.0-3.4	RCP-30-5A	5 mL
Rainbow Calibration, 8 peaks, 10^7/mL	3.0-3.4	RCP-30-20A	20 mL
Rainbow Calibration, 6 peaks, 10^7/mL	3.2 (+/-0.1)	RCP-32-5	5 mL
Rainbow Calibration, 4 peaks, 10^7/mL	3.5-4.0	RCP-35-5	5 mL
Rainbow Calibration, 6 peaks, 10^7/mL	6.0-6.4	RCP-60-5	5 mL

Figure 28 The dot plot below displays the bead distributions of the Rainbow Calibration Particles in FITC vs PE. (Catalog No. RCP-30-5A).



The **Rainbow Calibration Particles** (RCPs) contain a mixture of several similar size particles with different fluorescence intensities. Every particle contains a mixture of fluorophores that allows excitation at any wavelength from 365 to 650 nm. As a result, most channels in the flow cytometer can be calibrated using the same set of particles.

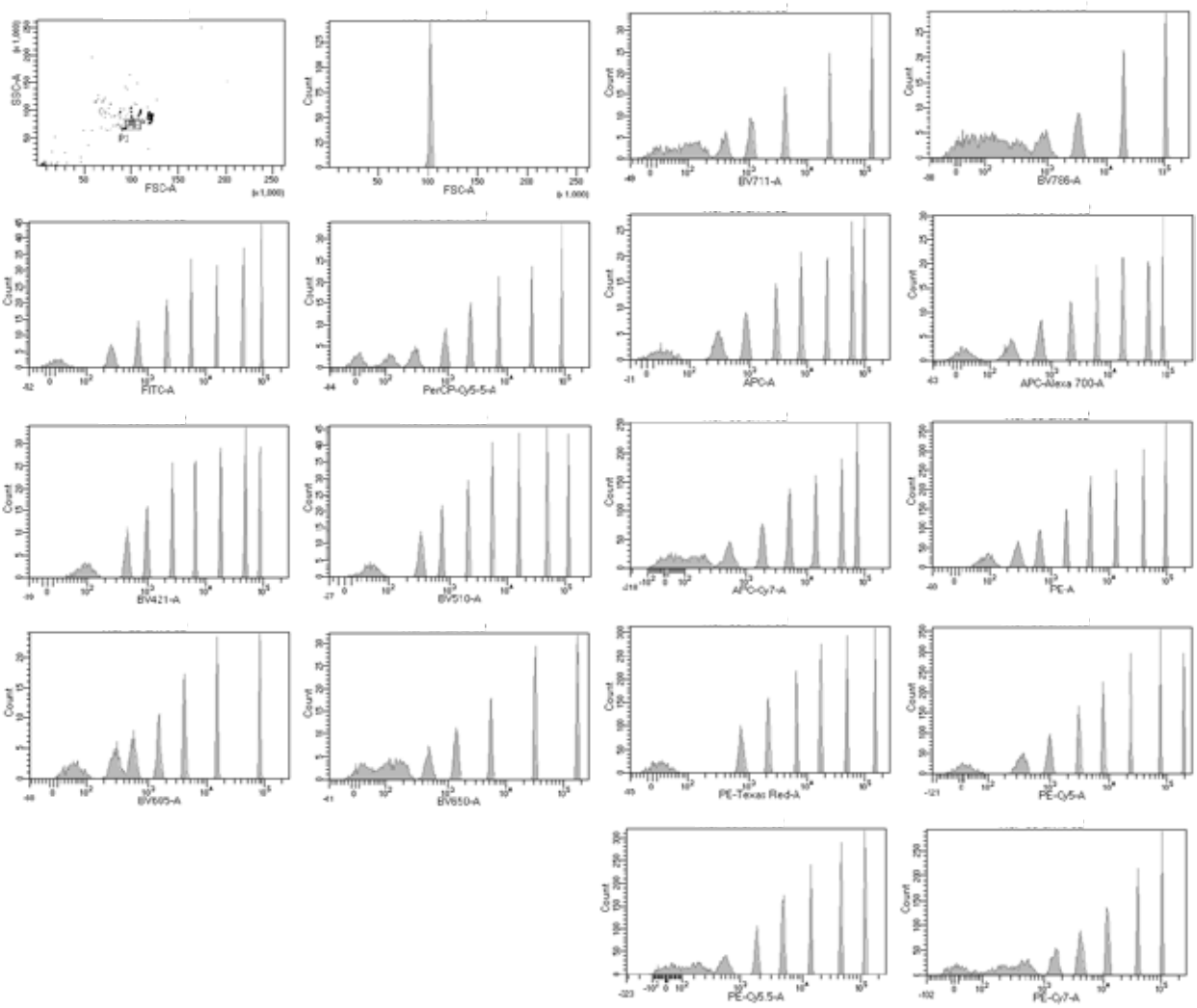
These particles are also used to determine the relative voltage range for each flow cytometry detector. This will determine the dynamic range of specific PMT detectors*.

*Perfetto, S. P., D. Ambrozak, et al. (2006). "Quality assurance for polychromatic flow cytometry." Nat. Protocols 1(3): 1522-1530.

The RCPs provide a reliable and reproducible particle mixture for calibrating flow cytometers. They are very stable since the fluorochromes are entrapped within the particles instead of being located on the surface. In addition, Spherotech uses fluorophores that are non-spectral matching to the commonly used fluorophores such as FITC, PE or PE-Cy5. As a result, the RCPs are stable in terms of fluorescence.

The RCPs are convenient and affordable to use for long term performance tracking or routine calibration. They are packaged in a dropper bottle to facilitate dispensing and storage. The diluted particles can be stored in the freezer for later use if desired to reduce costs. Dilution of a few drops of the particles from the dropper bottle to 1 mL of a diluent will provide adequate particle concentration for flow cytometer calibration. The diluted Rainbow Calibration Particles remain stable following repeated freezing and thawing.

Figure 29 Histograms showing individual peaks representing various fluorescence intensities in Rainbow Calibration Particles (Catalog No. RCP-30-5A, Lot No. AF02) on a BD Bioscience LSRFortessa™ X-20 are shown



New Selected References: Flow Cytometry Calibration Particles

- Vera S. Donnenberg, Albert D. Donnenberg, Coping with artifact in the analysis of flow cytometric data, Methods, Volume 82, 1 July 2015, Pages 3-11, ISSN 1046-2023, http://dx.doi.org/10.1016/j.ymeth.2015.03.012.(http://www.sciencedirect.com/science/article/pii/S1046202315001188) - Using RCP-30-5A to calibrated to predetermined photomultiplier target channels prior to each use using 8-peak Rainbow Calibration Particles
- Frankowski, M., Simon, P., Bock, N., El-Hasni, A, Schnakenberg, U., Neukammer, J. (2015) " Simultaneous optical and impedance analysis of single cells: A comparison of two microfluidic sensors with sheath flow focusing ". Eng. Life Sci. 15(3): 286-296 - Using RCP-30-5A to determine the stability of hydrodynamic focusing by measuring the coefficients of variations of calibration beads with specified size and fluorescence intensities.

Figure 30 Histograms showing individual peaks representing various fluorescence intensities in Rainbow Calibration Particles (Catalog No. RCP-30-5A, Lot No. AF02) on a Beckman Coulter CyAn™ ADP are shown below.

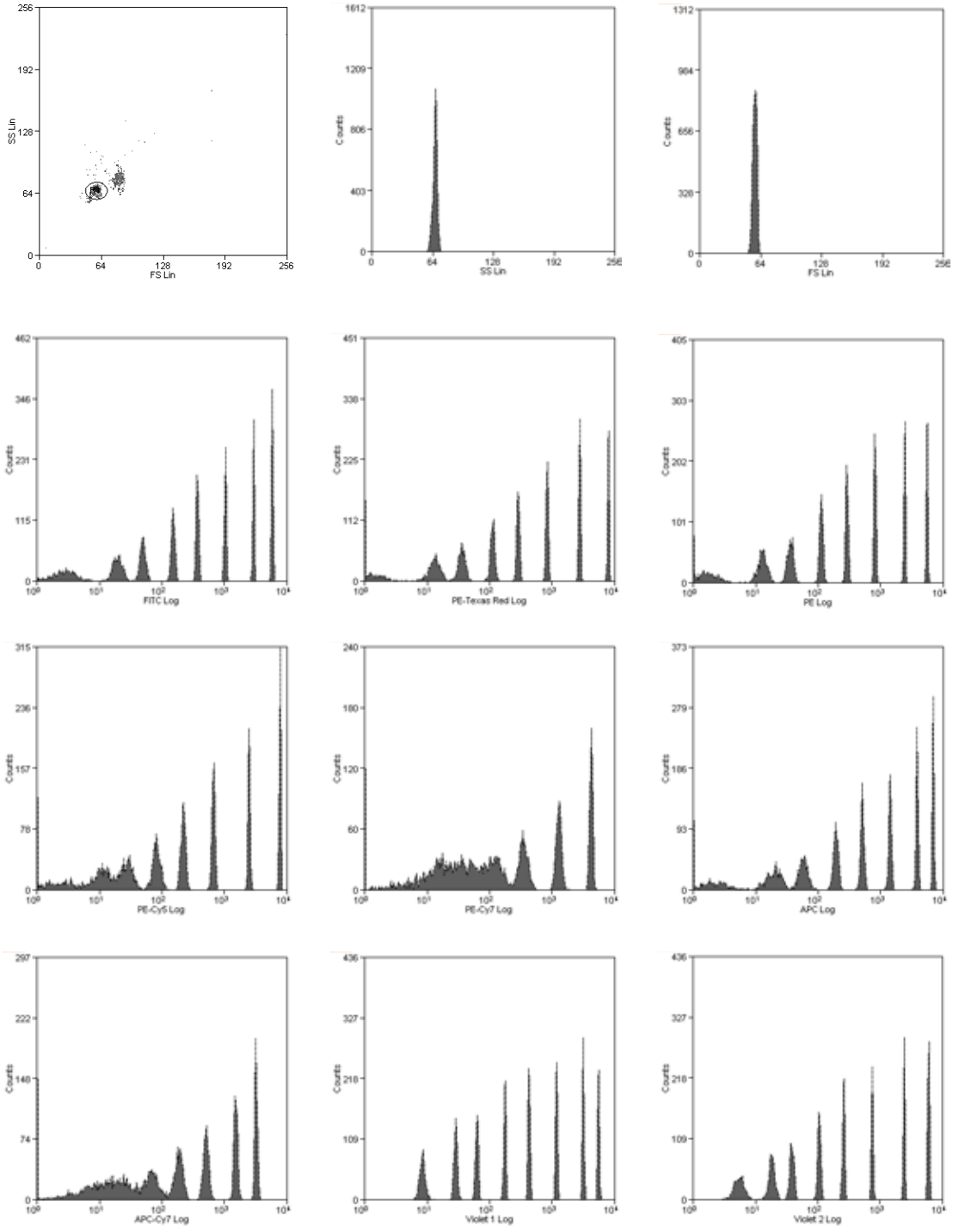
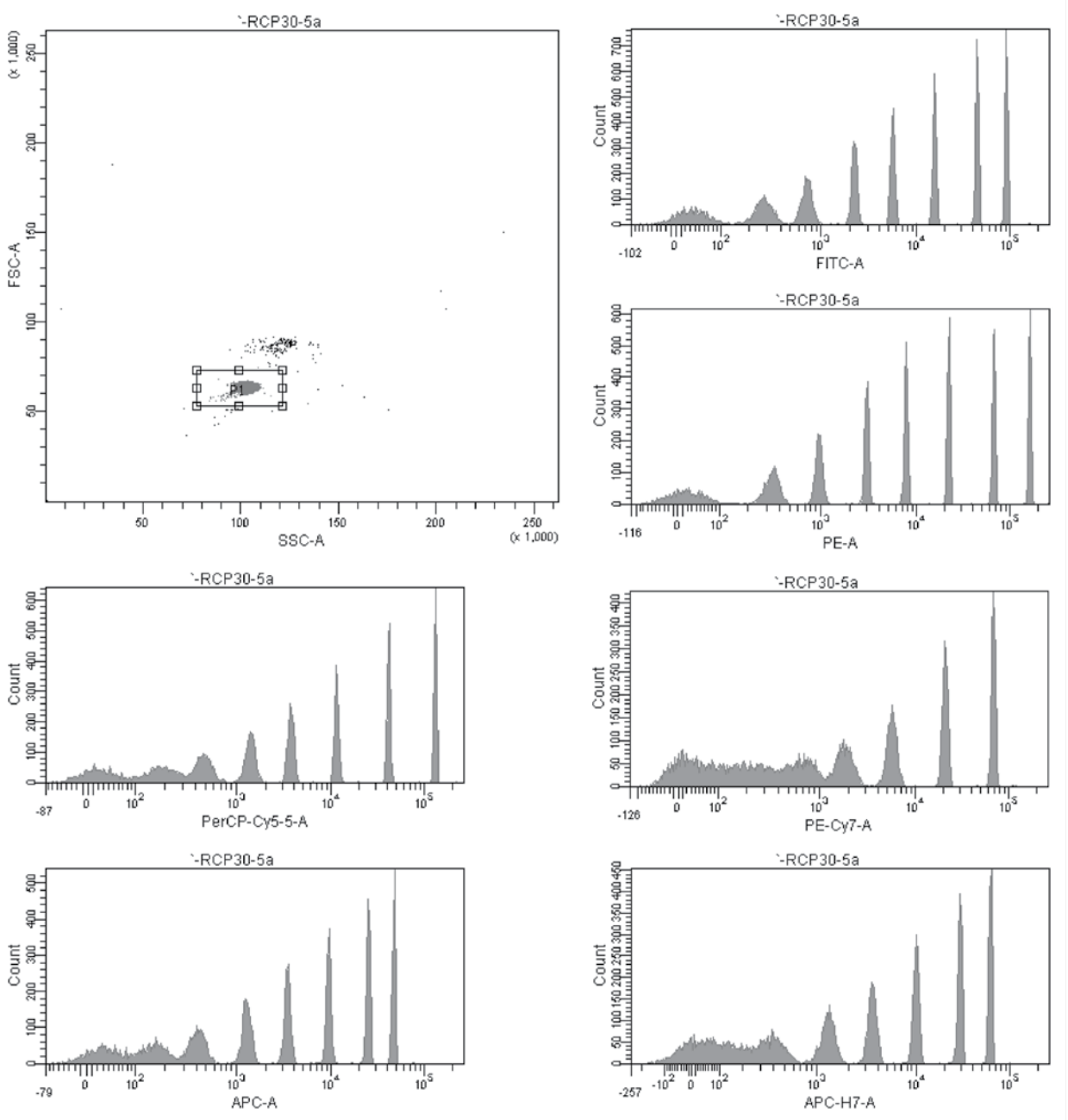
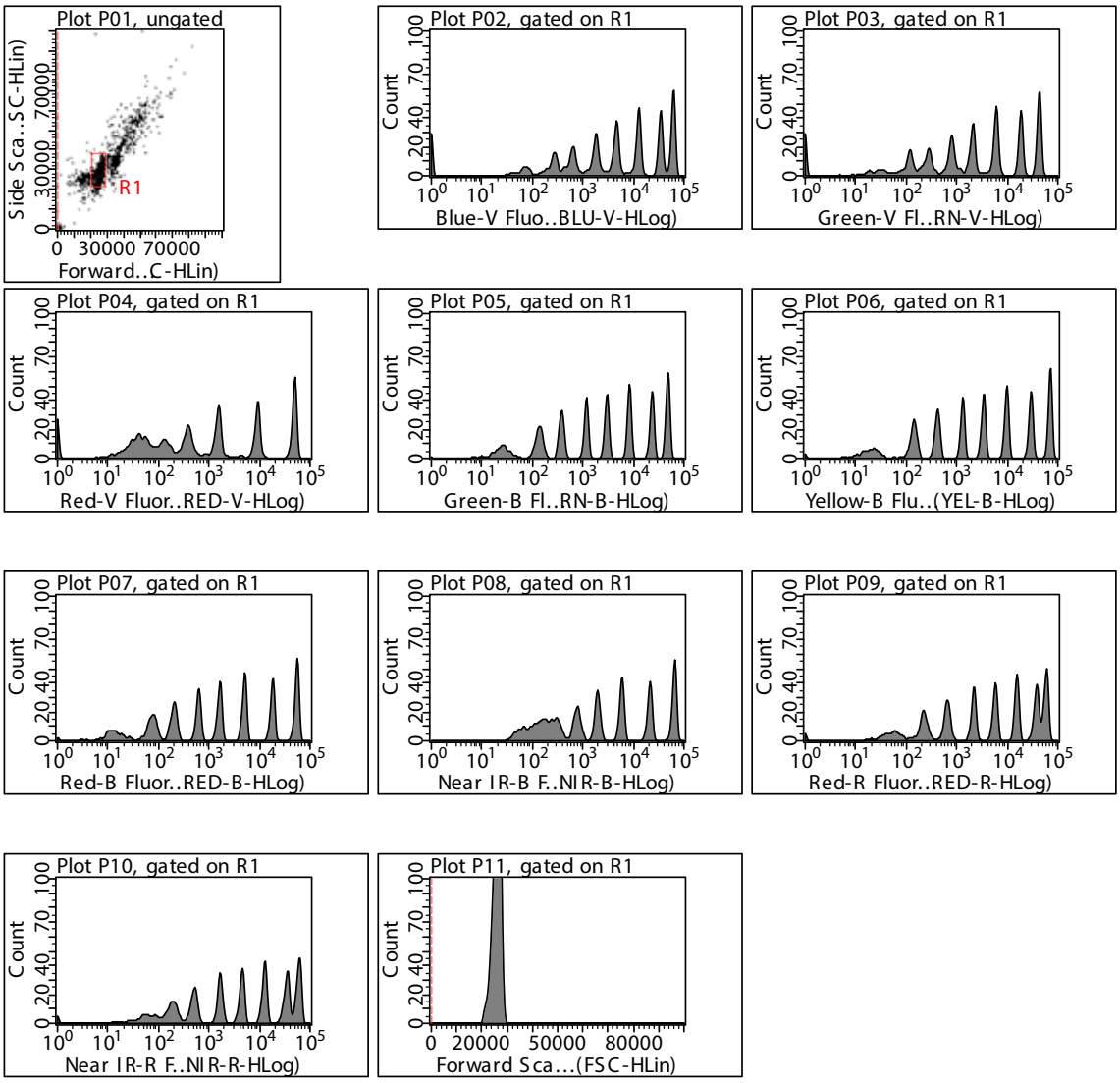


Figure 31 Histograms of the Rainbow Calibration Particles (Cat. No. RCP-30-5A, Lot No. AE01) on a BD FacsCanto™ II.



* Data provided by Laura Marszalek, Northwestern Memorial Hospital.

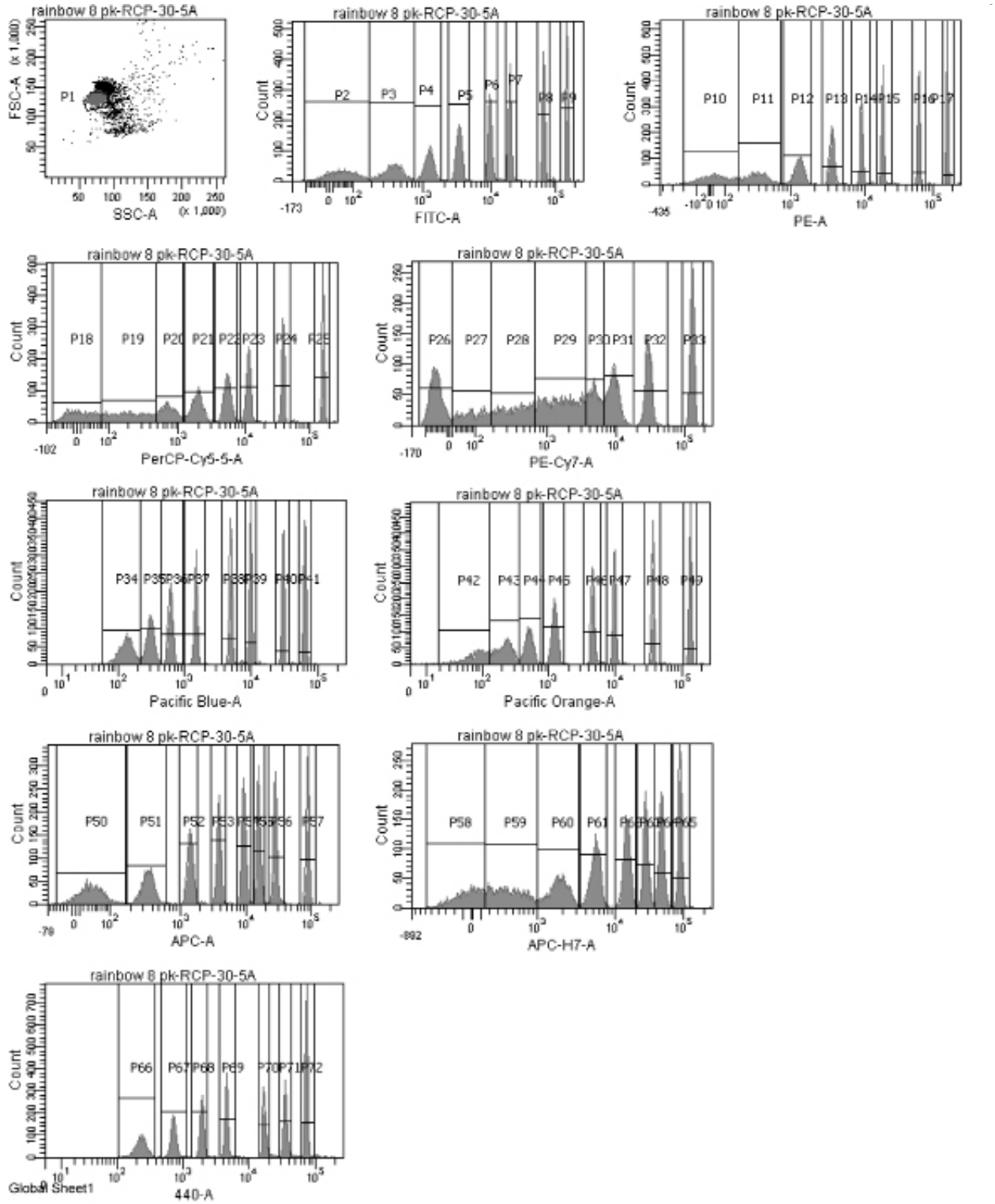
Figure 32 Histograms of the Rainbow Calibration Particles (Cat. No. RCP-30-5A, Lot No. AF01) on a Millipore guava easyCyte™ 12.



New Selected References: Flow Cytometry Calibration Particles

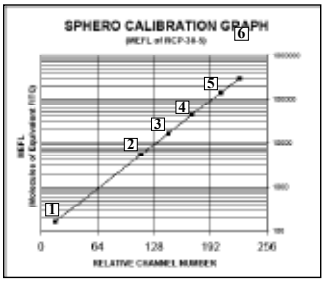
- Brown M, Stafford LJ, Onisk D, Joaquim T, Tobb A, et al. (2013) Snorkel: An Epitope Tagging System for Measuring the Surface Expression of Membrane Proteins. PLoS ONE 8(9): e73255. doi: 10.1371/journal.pone.0073255 - Flow cytometry was performed on a Guava EasyCyte Plus (Millipore) while calibration was performed using Rainbow Calibrator Particles Spherotech Cat. No. RCP 30-5A.

Figure 33 Histograms of the Rainbow Calibration Particles (Cat. No. RCP-30-5A, Lot No. AA01) on a BD LSR™ II.



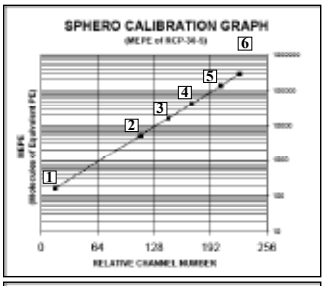
* Data provided by Laura Marszalek, Northwestern Memorial Hospital.

The relative number of fluorophores per particles has been determined for every peak of RCP-30-5 (Lot# AG01) in FL1 (FITC, MEFL), FL2 (RPE, MEPE), FL3 (RPE-Cy5, MEPCY) and FL4 (APC, MEAP) channels of flow cytometer to plot the calibration graph as shown below. The calibration graph is used to check the linearity of the PMT in each channel. In addition, the relative number of fluorophores can be cross calibrated with cells or particles stained with known number of spectral matching fluorophores such as FITC, PE, RPE-Cy5 to estimate the number of fluorophores on stained cells. The RCP-30-5A, which is identical to RCP-30-5 with the exception of two additional peaks between the blank and the dimmest peak of RCP-30-5 to give a total of 8 peaks is shown on Page 21. The RCP-30-5A is very useful in checking the sensitivity and resolution of the flow cytometer.



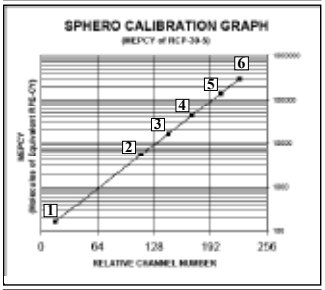
FITC Channel

PEAK	CH#	MEFL
1	26	-------
2	126	4447
3	158	14227
4	189	46322
5	218	133924
6	238	276897



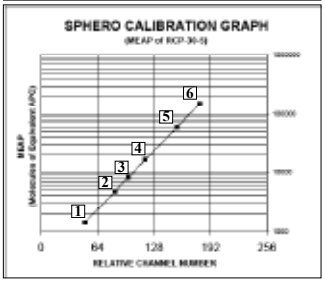
PE Channel

PEAK	CH#	MEPE
1	13	-------
2	119	3236
3	152	10754
4	184	34842
5	214	104483
6	237	245894



PE-CY5 Channel

PEAK	CH#	MECY
1	17	--------
2	108	8737
3	141	28177
4	176	93996
5	211	334087
6	243	1023447



APC Channel

PEAK	CH#	MEAP
1	23	--------
2	128	2395
3	161	8273
4	194	27652
5	221	75669
6	239	145428

A Template for MS Excel files, as shown below, is available free of charge upon request. The template will allow the user to check and report the linearity of PMT in all channels easily by using RCP-30-5, RCP-30-5A, RCP-60-5, URCP-38-2K, URCP-50-2K, RQC-4K or ACP-30-5K.

PMT LINEARITY QC RECORD

PEAK #	CH #	MEFL	MEFL LOG	CALC.	RESIDUAL	CALC. MEFL
1	23.98			1.958		91
2	82.63	792	2.899	2.898	0.15%	791
3	108.85	2079	3.318	3.319	0.25%	2083
4	139.92	6588	3.819	3.817	0.45%	6562
5	164.94	16471	4.217	4.218	0.37%	16531
6	193.56	47497	4.677	4.677	0.15%	47575
7	222.14	137049	5.137	5.136	0.23%	136680
8	240.75	271647	5.434	5.434	0.04%	271771
			Ave Residual		0.23%	
					Slope: 0.0160	
					Intercept: 1.5730	
					Rsq: 1.0000	

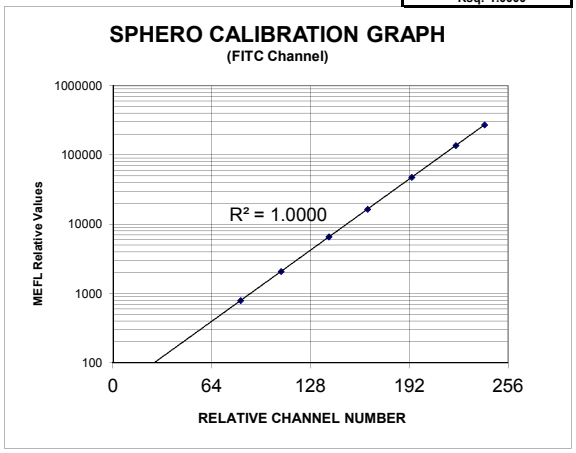


Figure 34 The Rainbow Calibration Particles are available in sizes of 2 to 6 µm to suit various applications. Additional histograms of Rainbow Calibration Particles are shown on Pages 21-29.

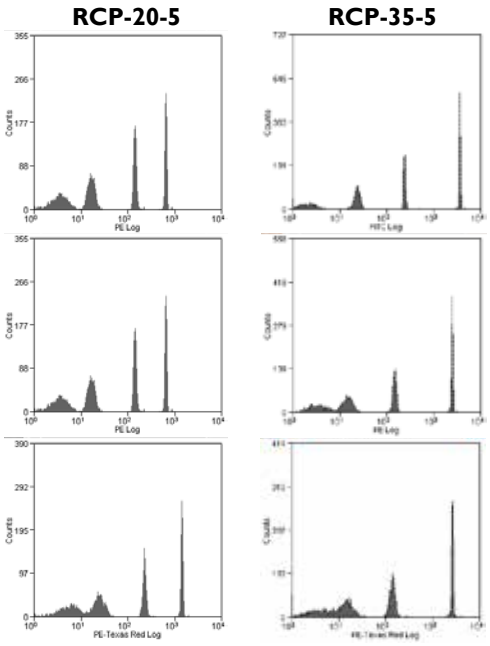


Figure 35 Histograms of the Rainbow Calibration Particles (Cat. No. RCP-60-5, Lot No. AF01).

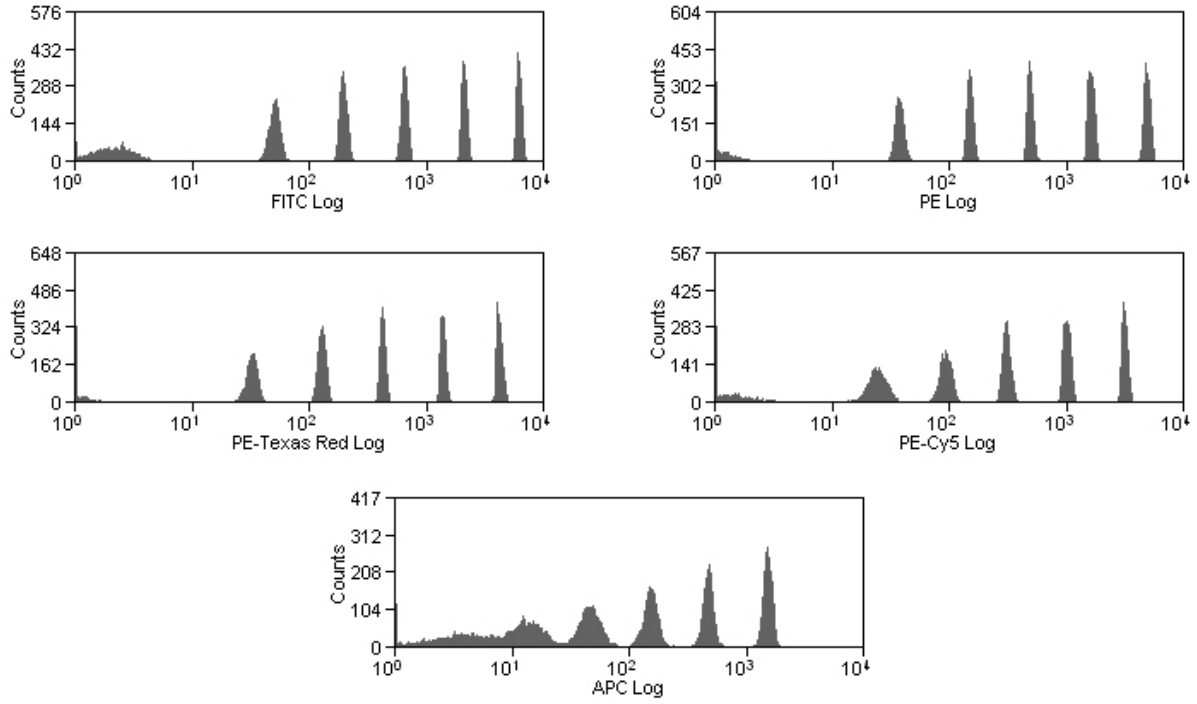


Figure 36 Histograms of the Rainbow Calibration Particles (Cat. No. RCP-32-5, Lot No. AF02).

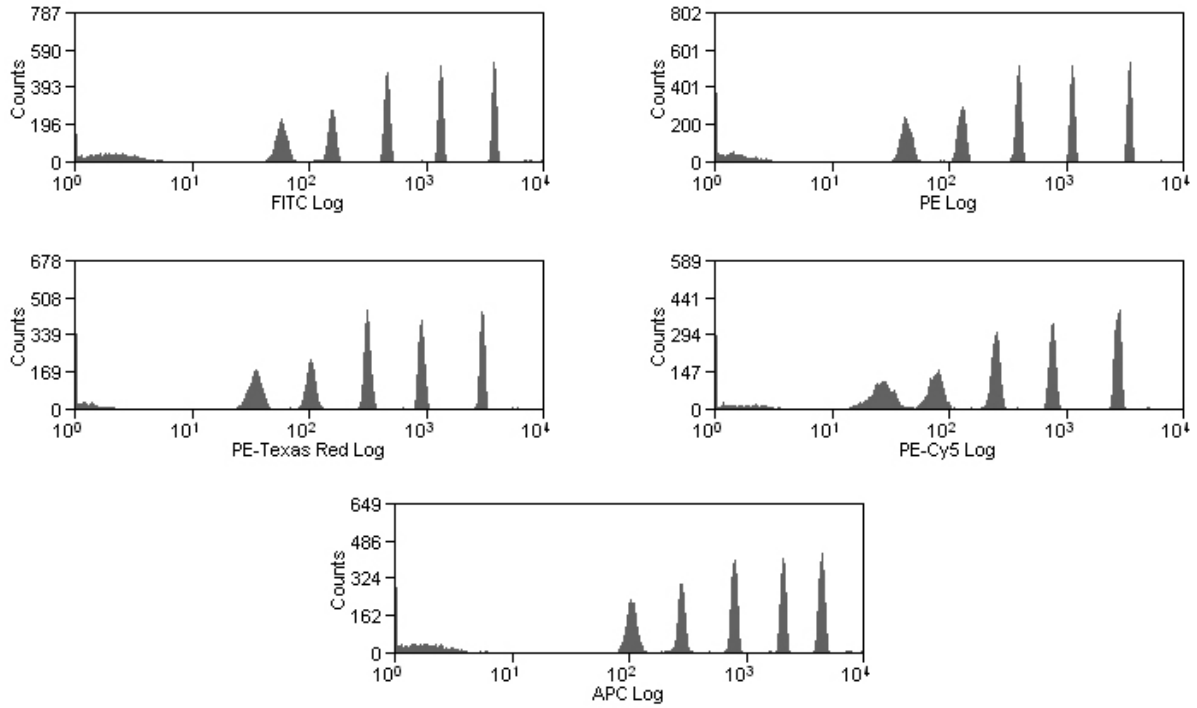
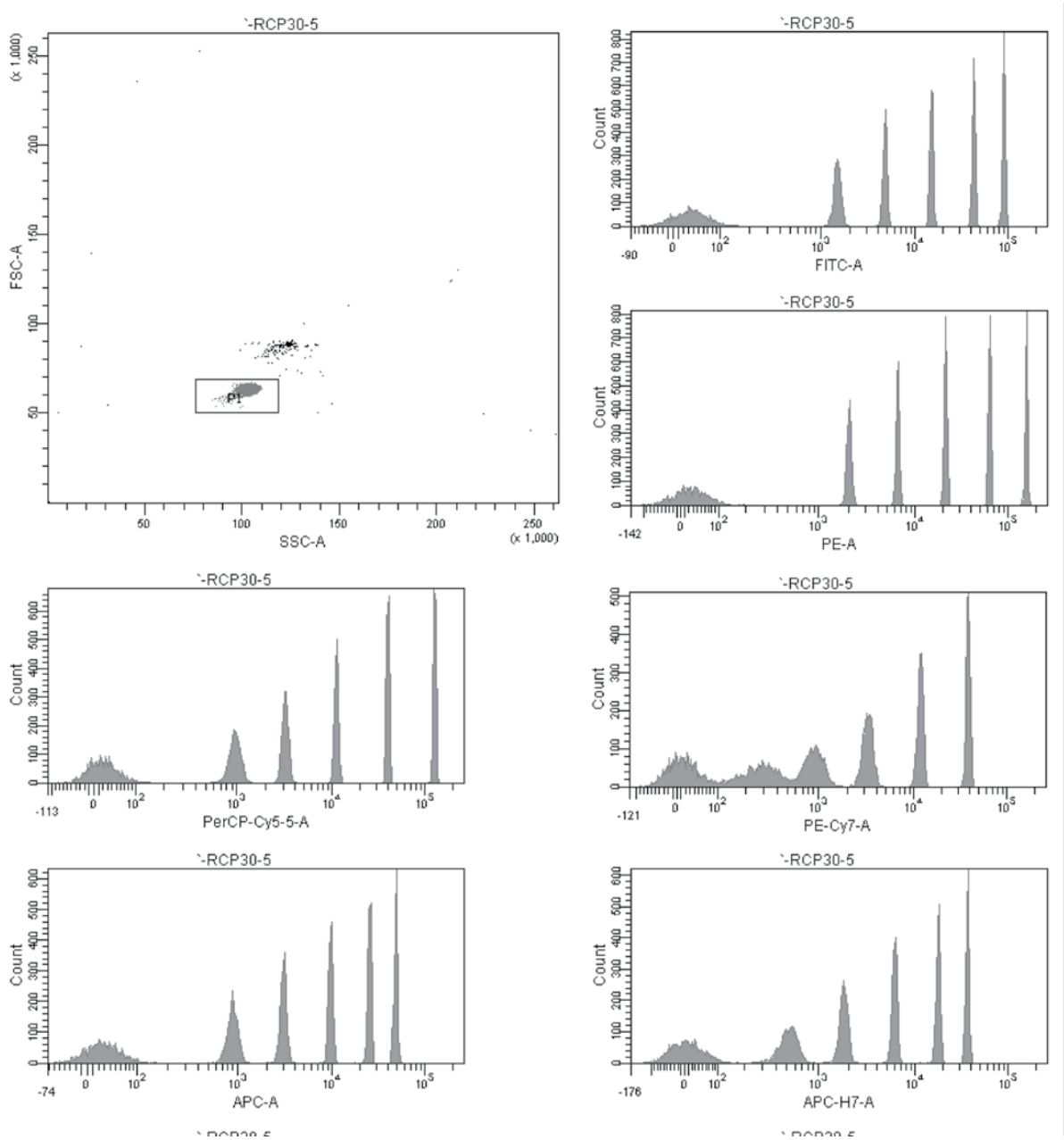
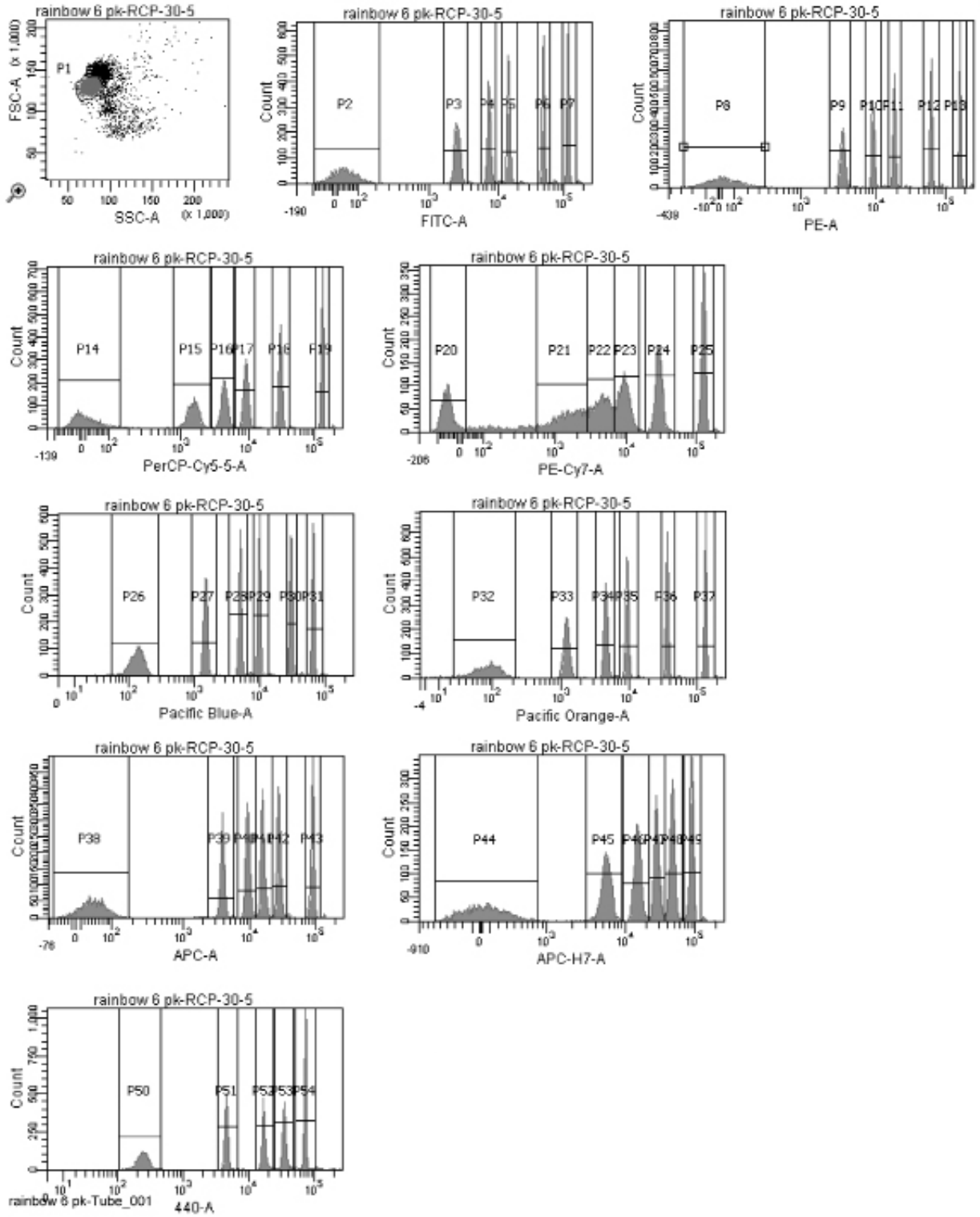


Figure 37 Histograms of the Rainbow Calibration Particles (Cat. No. RCP-30-5, Lot No. AD03) on a BD FacsCanto™ II.



* Data provided by Laura Marszalek, Northwestern Memorial Hospital.

Figure 38 Histograms of the Rainbow Calibration Particles (Cat. No. RCP-30-5, Lot No. AA01) on a BD LSR™ II.



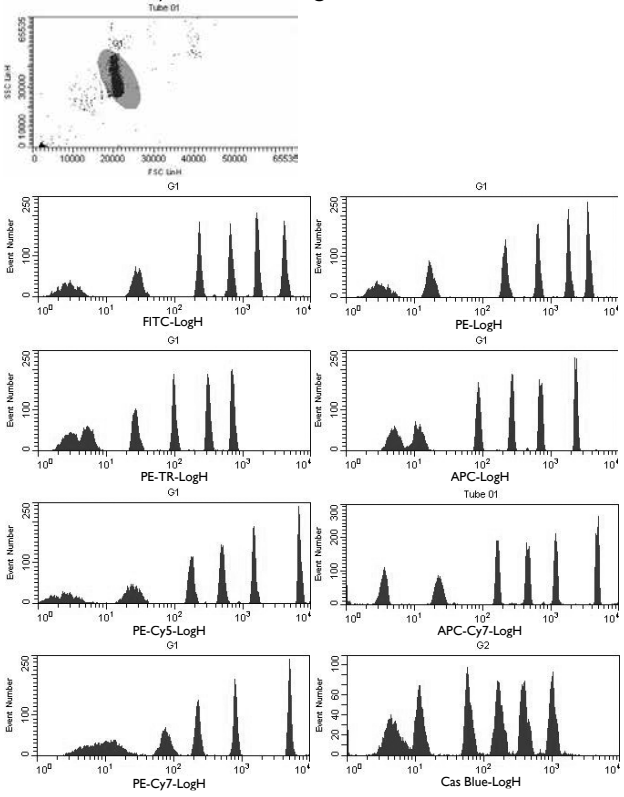
* Data provided by Laura Marszalek, Northwestern Memorial Hospital.

SPHERO™ Ultra Rainbow Calibration Particle Kits

- Consists of one bead with multiple fluorophores and intensities for calibration in all channels of the flow cytometer
- Designed for routine calibration and long term performance tracking
- Used to optimize the linearity, resolution and sensitivity
- Simplifies the calibration in the UV, Violet, Far Red, and IR channels.

A new set of calibration particles, **Ultra Rainbow Calibration Particles**, are now available for performance tracking of flow cytometers with fluorescent channels in the Far Red. The **Ultra Rainbow Calibration Particle Kits** are available in either 3.8 µm, or 5.1 µm. These particles are supplied as a kit. One 3mL bottle of blank particles and a 2 mL bottle of fluorescent particles. The **Ultra Rainbow Calibration Particles** have improved resolution in the PE-Cy7, APC, and APC-Cy7 channels.

Figure 39 Histograms of the Ultra Rainbow Calibration Particles (Cat. No. URCP-38-2K, Lot No. AG01) on a Stratedigm S1400.



Particle Type and Surface	Size, µm	Catalog No.	Unit
Ultra Rainbow Calibration Kit, 6 peaks, 10^7/mL	3.5-3.9	URCP-38-2K	2 mL
Ultra Rainbow Calibration Kit, 6 peaks, 10^7/mL	3.5-3.9	URCP-38-20K	20 mL
Ultra Rainbow Calibration Kit, 6 peaks, 10^7/mL	5.0-5.4	URCP-50-2K	2 mL
Ultra Rainbow Calibration, 3 peaks, 10^6/mL	3.0-3.4	URCP01-30-10K	10x3mL
Ultra Rainbow Calibration, 6 peaks, 10^7/mL	8.0-12.9	URCP-100-2	2 mL

Figure 40 Histograms of the Ultra Rainbow Calibration Particles (Cat. No. URCP-38-2K, Lot No. AG01) on a Beckman Coulter Cyan™ ADP.

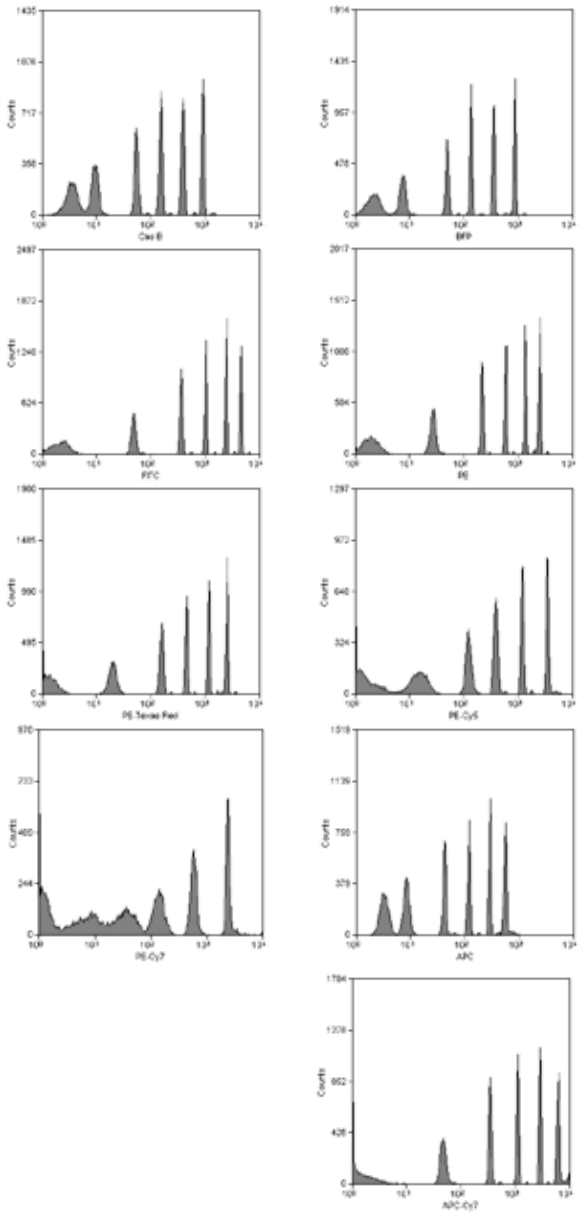


Figure 41 Histograms showing individual peaks representing various fluorescence intensities in Rainbow Calibration Particles (Catalog No. URCP-38-2K, Lot No. AG01) on a BD Bioscience LSRFortessa™ X-20 are shown

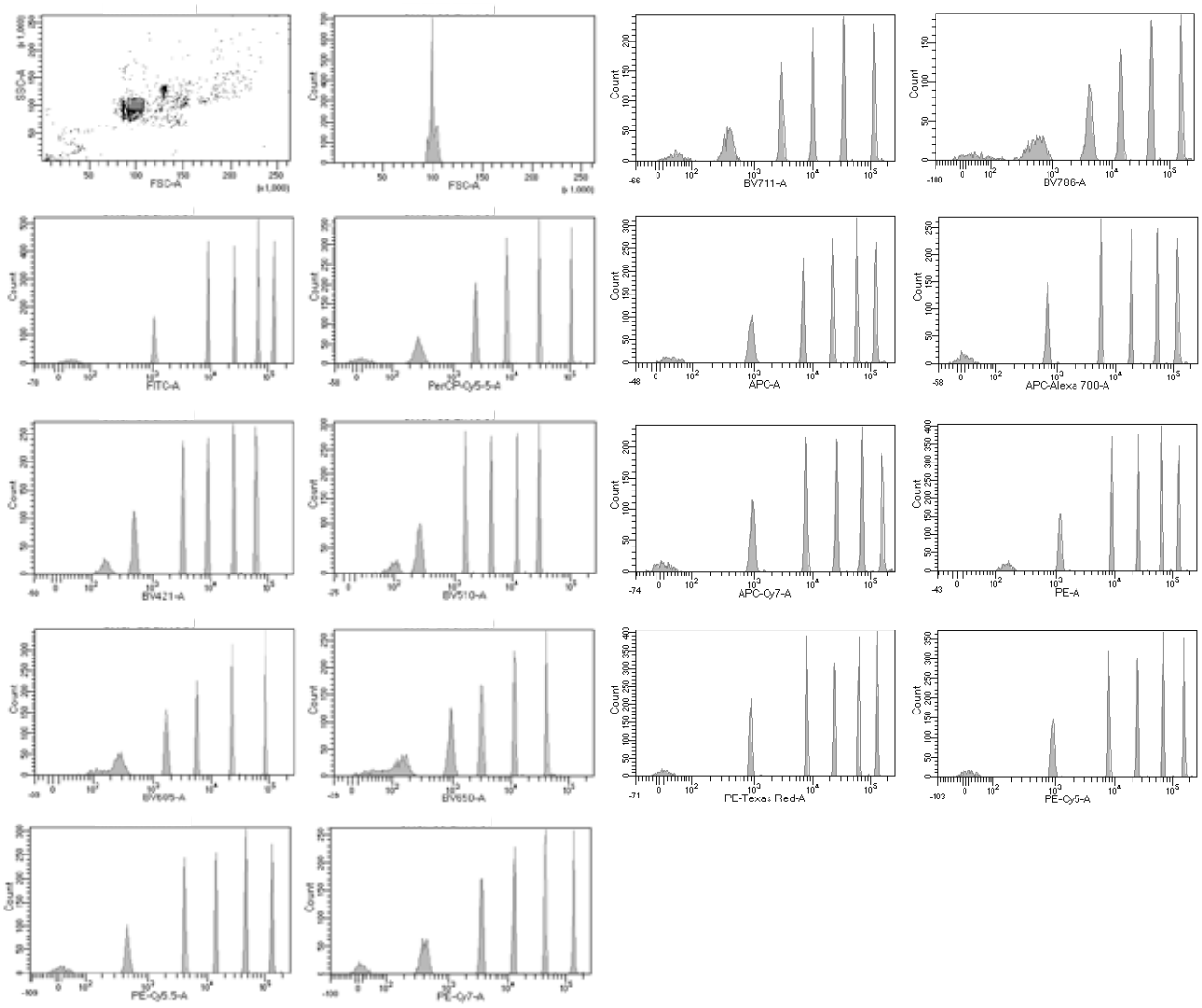


Figure 42 Histograms showing individual peaks representing various fluorescence intensities in Rainbow Calibration Particles (Catalog No. URCP-50-2K, Lot No. AG01) on a BD Bioscience LSRFortessa™ X-20 are shown

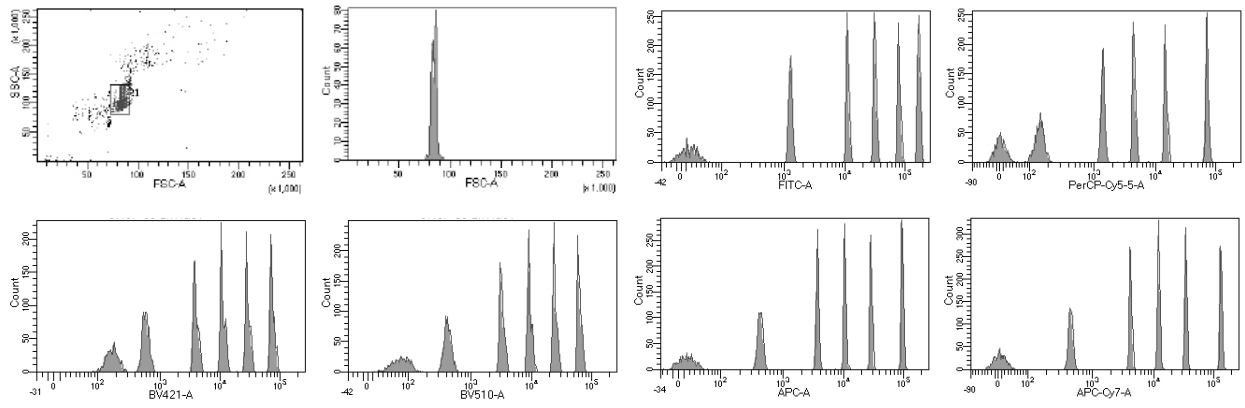
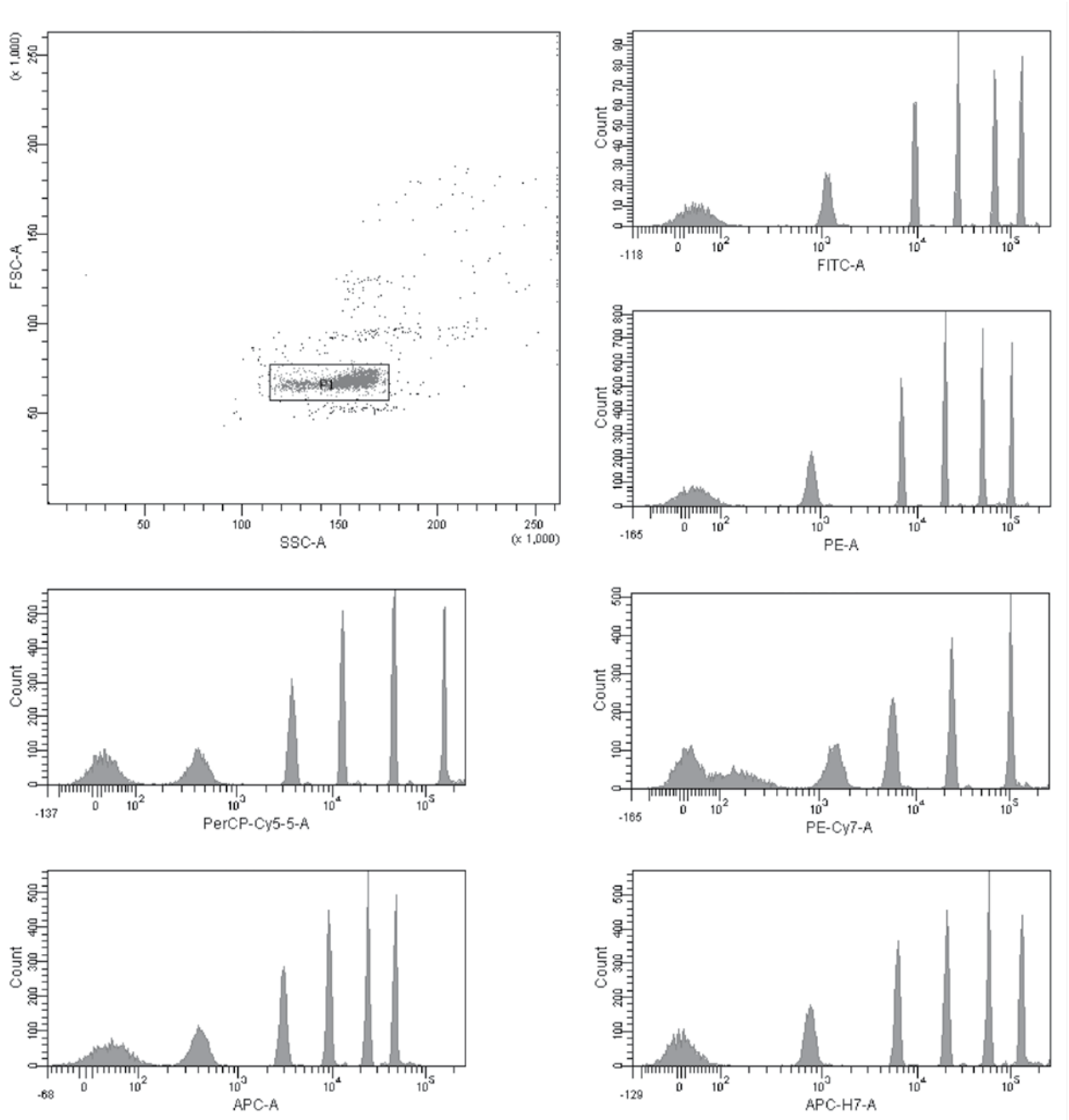
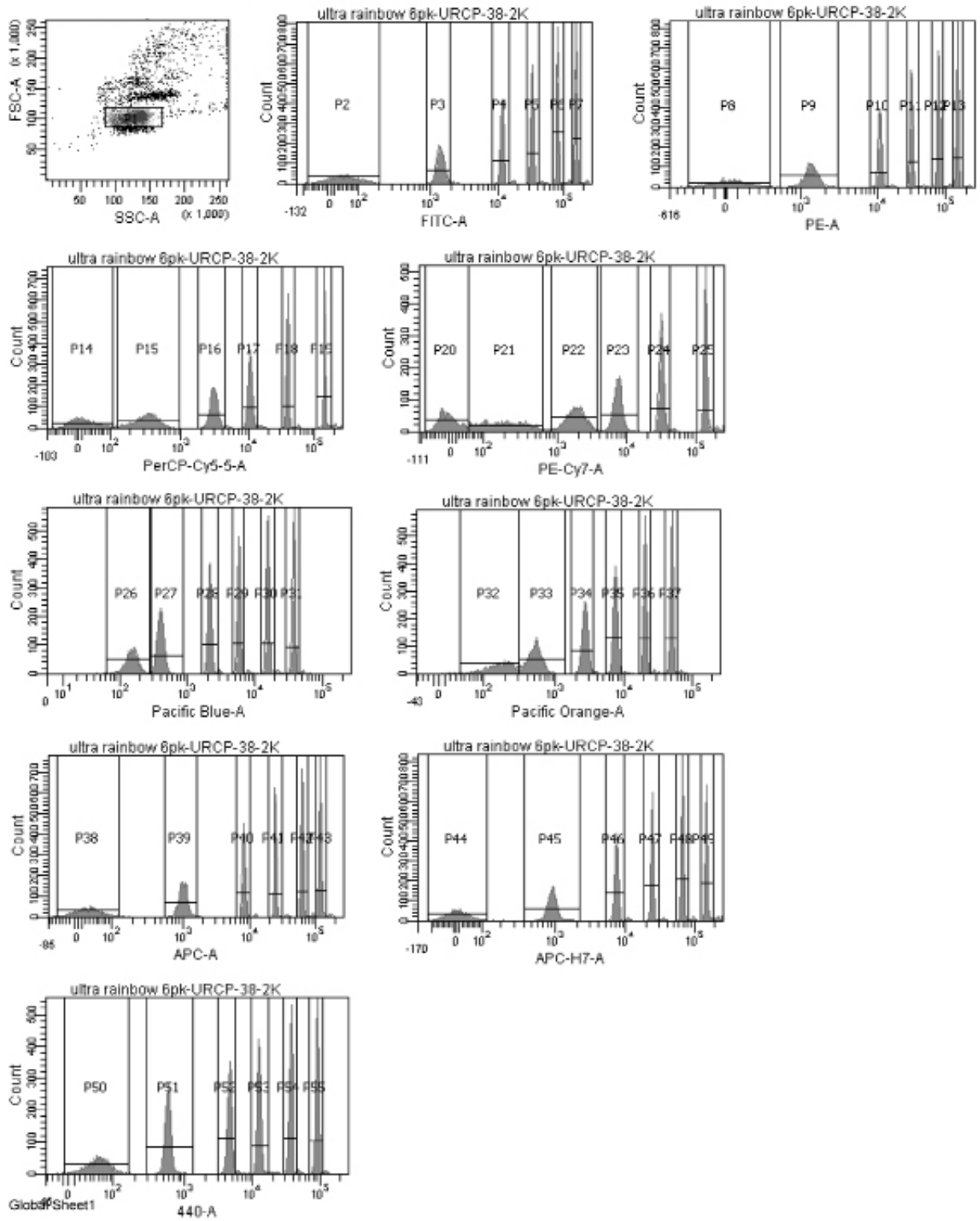


Figure 43 Histograms of the Ultra Rainbow Calibration Particles (Cat. No. URCP-38-2K, Lot No. AD03) on a BD FacsCanto™ II.



* Data provided by Laura Marszalek, Northwestern Memorial Hospital.

Figure 44 Histograms of the Ultra Rainbow Calibration Particles (Cat. No. URCP-38-2K, Lot No. AD03) on a BD LSR™ II.



* Data provided by Laura Marszalek, Northwestern Memorial Hospital.

Figure 45 Histograms of the Ultra Rainbow Calibration Particles (Cat. No. URCP-38-2K, Lot No. AG01) on a Millipore easyCyte12.

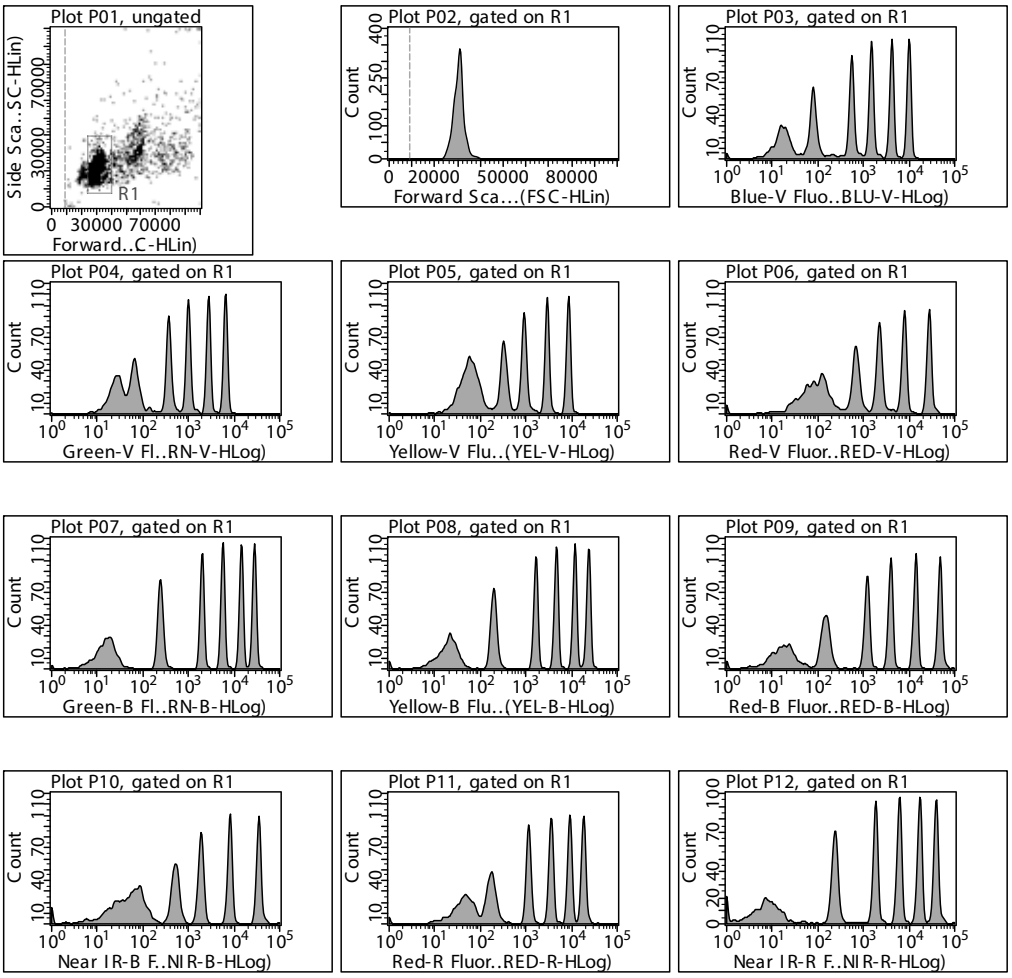
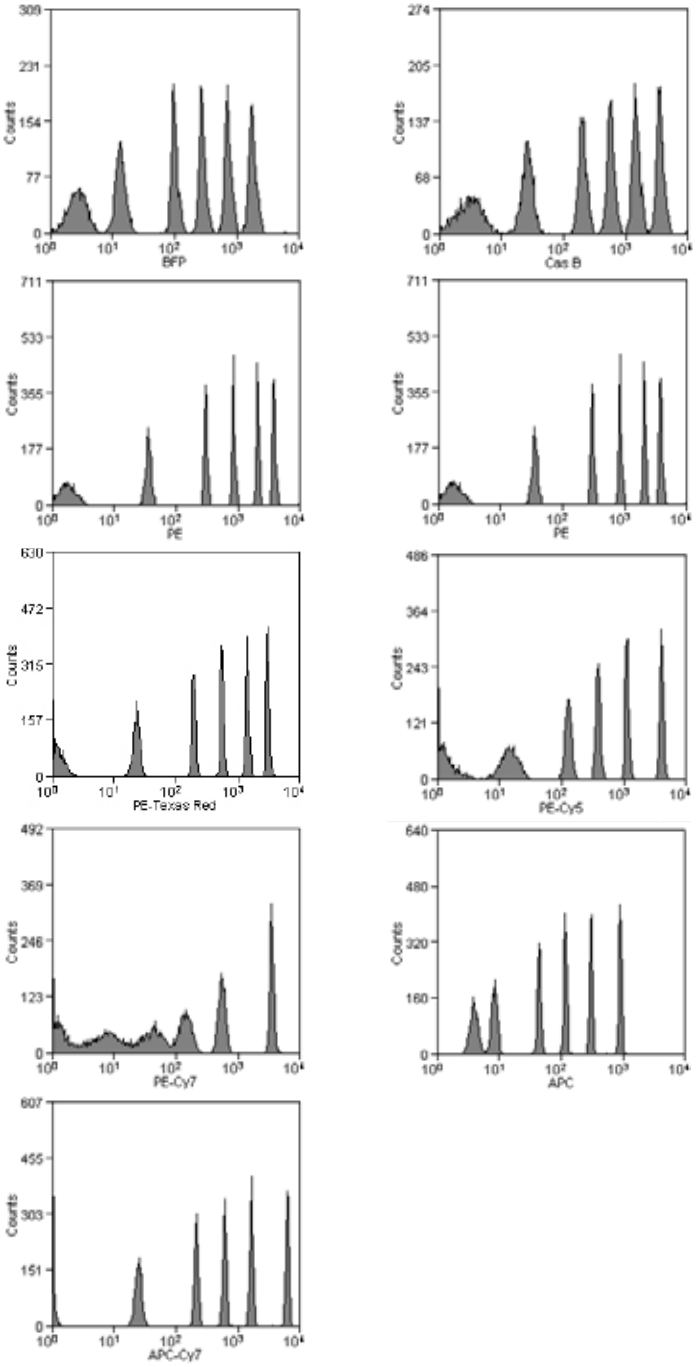
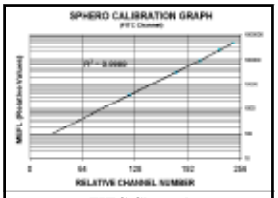


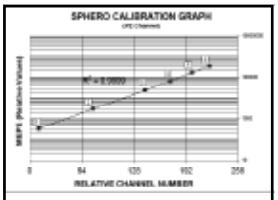
Figure 46 Histograms of the Ultra Rainbow Calibration Particles (Cat. No. URCP-50-2K, Lot No. AG01) on a Beckman Coulter Cyan™ ADP.





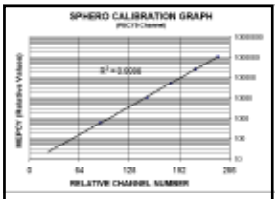
FITC Channel

PEAK	CH#	MEFL
1	28	
2	121	2615
3	178	25590
4	205	63305
5	229	155800
6	248	328880



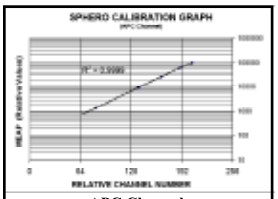
PE Channel

PEAK	CH#	MEPE
1	13	
2	89	2205
3	148	19085
4	177	54450
5	201	134910
6	218	248895



PE-CY5 Channel

PEAK	CH#	MECY
1	8	
2	65	1270
3	122	11790
4	152	37800
5	183	127280
6	225	618430



APC Channel

PEAK	CH#	MEAP
1	66	
2	83	6780
3	137	56850
4	165	160850
5	188	403930
6	210	919070

SPHERO™ Easy Calibration Fluorescent Particles

- Surface labeled polystyrene beads with commonly used fluorophores
- Used to determine the linearity of the logarithmic amps and sensitivity of flow cytometers in specific channels
- Supplied as individual products for use in specific channels of flow cytometers.

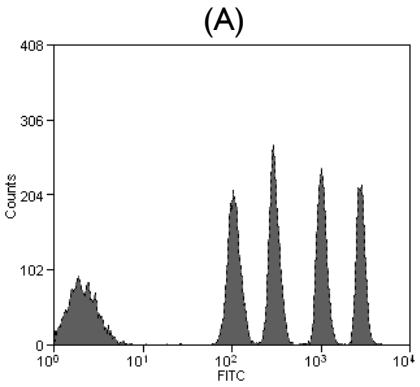
The **Easy Calibration Fluorescent Particles** consist of a mixture of particles with intensities calibrated in terms of Molecules of Equivalent Fluorochrome (MEF) units. Spherotech offers **Easy Calibration Fluorescent Particles** with three different fluorochromes including FITC, PE, PE-Cy5 and GFP.

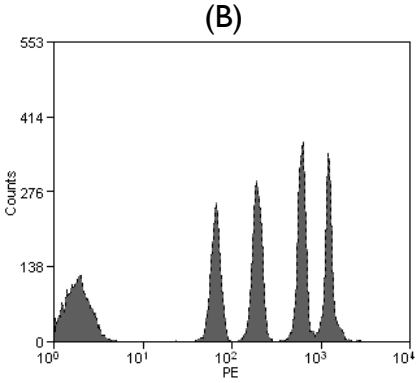
Since each **Easy Calibration Fluorescent Particle** population is assigned a MEF unit, linearity calibrations and quantitative determinations can be performed. A linearity calibration is performed by graphing each population's channel number against its assigned MEF value. As a result, a regression line is created. This line can be used to track the performance history of the instrument. In addition, quantitative determinations for stained cells is performed when their obtained channel numbers are cross calibrated against the regression line. Refer to **SPHERO™** Technical Note #9 (STN #9) at *http://www.spherotech.com/tech.htm* for more information regarding the cross calibration of unknowns.

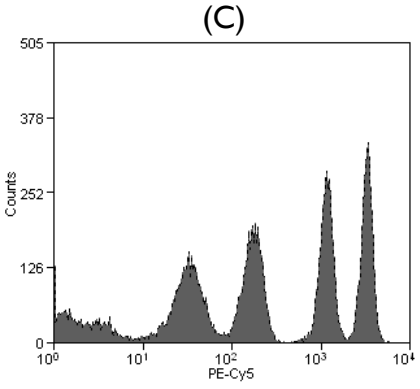
Unlike the Rainbow Calibration Particles, these beads are surfaced labeled. As a result, these beads interact with their environment and are used to detect changes within the flow cytometer such as contaminants or pH changes.

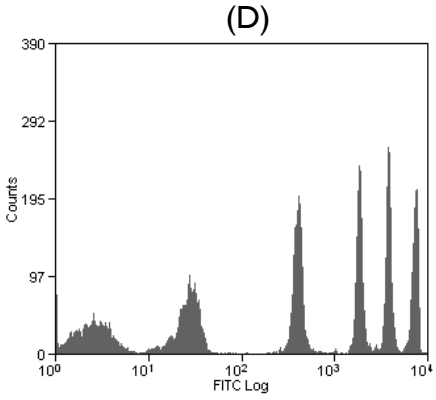
Particle Type and Surface	Size, µm	Catalog No.	Unit
FITC Calibration , 2x10^6/mL, 5 peaks	3.0-3.4	ECFP-F1-5K	5 x 1 mL
PE Calibration , 2x10^6/mL, 5 peaks	3.0-3.4	ECFP-F2-5K	5 x 1 mL
PE-Cy5 Calibration , 2x10^6/mL, 5 peaks	3.0-3.4	ECFP-F4-5K	5 x 1 mL
GFP Calibration , 2x10^6/mL, 6 peaks	3.0-3.4	ECFP-F9-5K	6 x 1 mL

Figure 47 Histograms of the Easy Calibration Fluorescent Particles; (A) Cat. No. ECFP-F1-5K (FITC), (B) Cat. No. ECFP-F2-5K (PE), (C) Cat. No. ECFP-F4-5K (Pe-Cy5), and (D) Cat. No. ECFP-F9-5K (GFP) on a Beckman Coulter Cyan™ ADP.







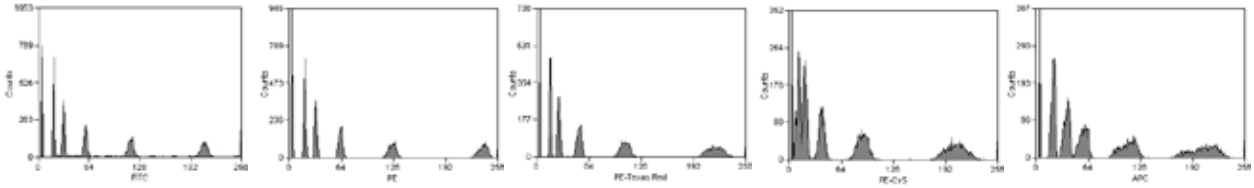


SPHERO™ Rainbow Linear Calibration Particles

- Designed to check the linearity of the PMT in the linear scale of flow cytometers
- Used to quantitate the DNA content of cells stained with either Hoechst dyes, DAPI, Ethidium Bromide, Propidium Iodide, YOYO or TOTO.

Particle Type and Surface	Size, µm	Catalog No.	Unit
Rainbow Linear Calibration, 10^7/mL, 6 peaks	3.0-3.4	RLP-30-5	5 mL

Figure 48 Histograms of the Rainbow Linear Calibration Particles (Cat. No. RLP-30-2, Lot No. AE01) on a Beckman Coulter Cyan™ ADP.

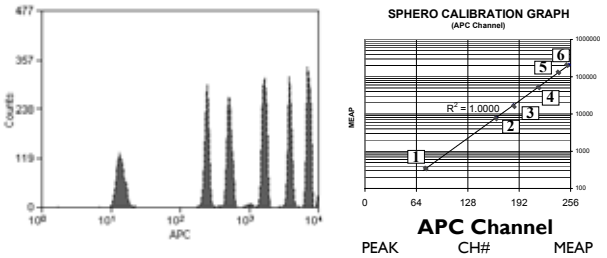


SPHERO™ Allophycocyanin Calibration Particles

- Contain a mixture of fluorescent particles with different intensities in the Allophycocyanin channel
- Excited with either He-Ne laser at 632 nm or a diode laser at 635 nm
- Used to determine the linearity of the logarithmic amps and sensitivity of flow cytometers in Allophycocyanin channel.

Particle Type and Surface	Size, µm	Catalog No.	Unit
APC Calibration, 10^7/mL, 6 peaks	3.0-3.4	ACP-30-5K	2x5 mL

Figure 49 Histograms of the Allophycocyanin Calibration Particles Kit (Cat. No. ACP-30-5K, Lot No. AC01) on a Beckman Coulter Cyan™ ADP.



APC Channel

PEAK	CH#	MEAP
1	73	336
2	153	7880
3	174	17400
4	206	53100
5	230	130000
6	247	208000

SPHERO™ IR Calibration Particles

- Contain a mixture of fluorescent particles with different intensities in IR channels
- Excited with 785-nm IR diode laser or other laser operating in the infrared
- Used to determine the linearity of the logarithmic amps and sensitivity of flow cytometers in near-IR channels.

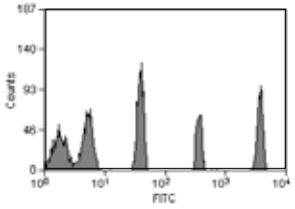
Particle Type and Surface	Size, µm	Catalog No.	Unit
IR Calibration, 10^7/mL, 800nm EM, 4 peaks	3.5 (+/- 0.2)	FCP-3580-2	2 mL

SPHERO™ Yellow Calibration Particles

- Designed for routine calibration of the Fluorescein channel
- Contains a set of Yellow Particles with different fluorescence intensities.
- Increased stability over FITC surface labeled particles.

Particle Type and Surface	Size, µm	Catalog No.	Unit
Yellow Calibration, 10^7/mL, 6 peaks	6.0-8.0	YCP-70-5	5 mL

Figure 50 Histograms of the Yellow Calibration Particles (Cat. No. YCP-70-2, Lot No. AE01) on a Beckman Coulter Cyan™ ADP.

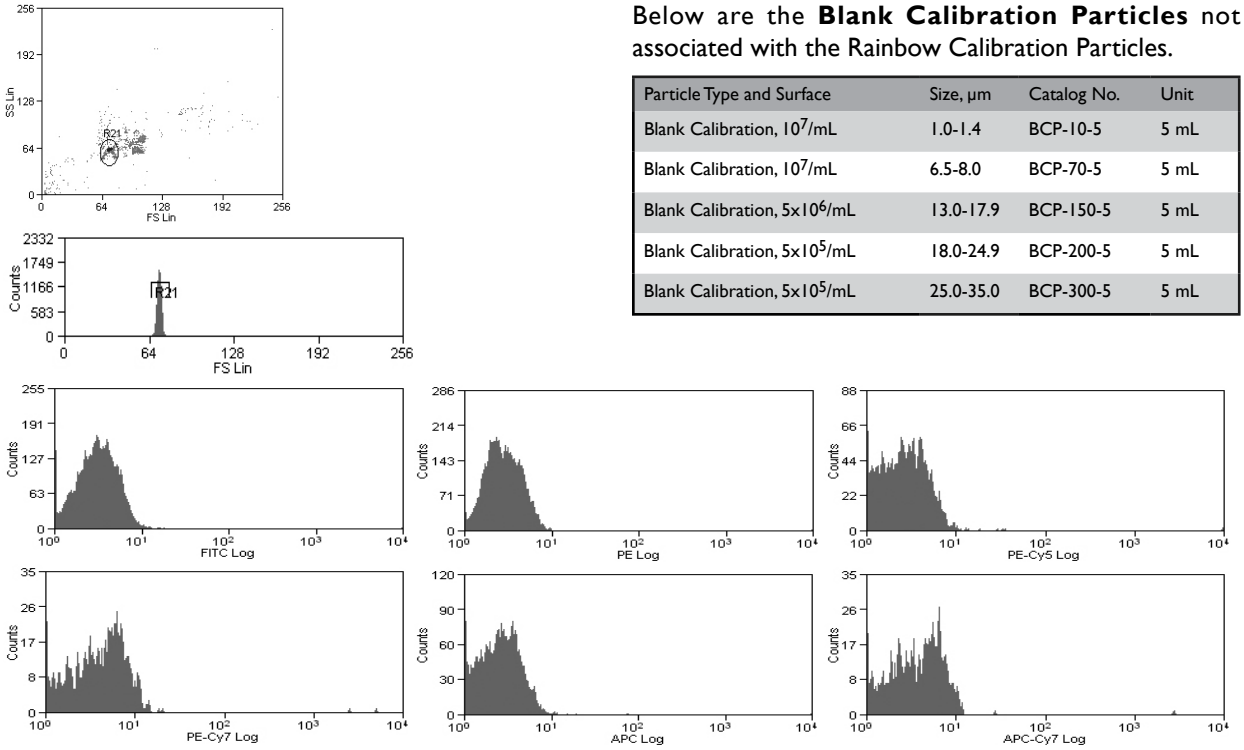


SPHERO™ Blank Calibration Particles

- Consists of the blank peak of the corresponding Rainbow Calibration Particles
- Used to set the threshold of the instrument in each channel.

Particle Type and Surface	Size, µm	Catalog No.	Unit
Blank Calibration, 10^7/mL	1.8-2.2	BCP-20-5	5 mL
Blank Calibration, 10^7/mL	3.0-3.4	BCP-30-5	5 mL
Blank Calibration, 10^7/mL	3.2 (+/-0.1)	BCP-32-5	5 mL
Blank Calibration, 10^7/mL	3.5-4.0	BCP-35-5	5 mL
Blank Calibration, 10^7/mL	5.0-5.9	BCP-50-5	5 mL
Blank Calibration, 10^7/mL	6.0-6.4	BCP-60-5	5 mL
Blank Calibration, $5x10^6$/mL	8.0-12.9	BCP-100-5	5 mL

Figure 51 Histograms of the Blank Calibration Particles (Cat. No. BCP-30-5, Lot No. AG01) on a Beckman Coulter Cyan™ ADP.



Below are the **Blank Calibration Particles** not associated with the Rainbow Calibration Particles.

Particle Type and Surface	Size, µm	Catalog No.	Unit
Blank Calibration, 10^7/mL	1.0-1.4	BCP-10-5	5 mL
Blank Calibration, 10^7/mL	6.5-8.0	BCP-70-5	5 mL
Blank Calibration, $5x10^6$/mL	13.0-17.9	BCP-150-5	5 mL
Blank Calibration, $5x10^5$/mL	18.0-24.9	BCP-200-5	5 mL
Blank Calibration, $5x10^5$/mL	25.0-35.0	BCP-300-5	5 mL

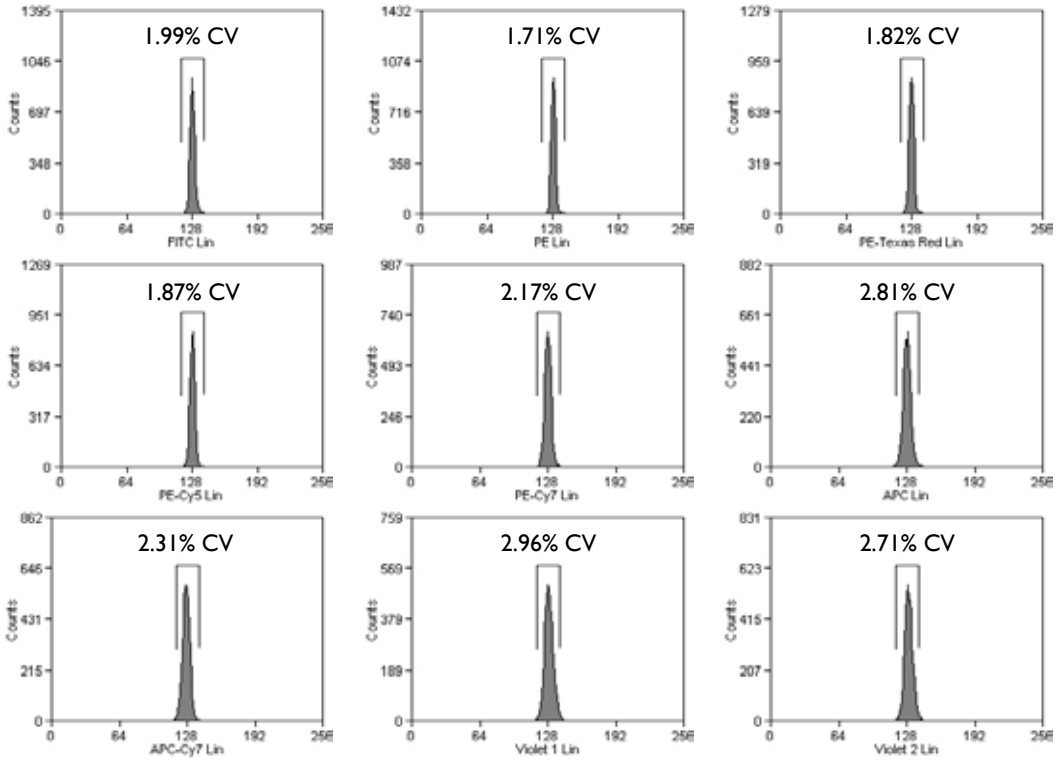
SPHERO™ Ultra Rainbow Fluorescent Particles

- Consists of a single peak for optical alignment of any flow cytometer in all channels from UV to Far Red
- Determines if the flow cell is clean and without fluidic blockage
- Measures the coefficients of variation (CVs), peak channels, and histogram distribution to determine the functionality of flow cytometers.

New flow cytometers, with fluorescent channels from the UV to Far Red, and corresponding fluorescent conjugates are now available. As a result, we have developed the Ultra Rainbow Fluorescent Particles with enhanced UV and Far Red fluorescence intensity. The Ultra Rainbow Fluorescent Particles contain a single peak and are designed for checking the optical alignment of any flow cytometer in all channels.

Particle Type and Surface	Size, µm	Catalog No.	Unit
Ultra Rainbow Fluorescent, 10^{10}/mL	0.1-0.3	URFP-02-5	5 mL
Ultra Rainbow Fluorescent, 10^{7}/mL	0.4-0.6	URFP-05-5	5 mL
Ultra Rainbow Fluorescent, 10^{7}/mL	1.0-1.4	URFP-10-5	5 mL
Ultra Rainbow Fluorescent, 10^{7}/mL	3.0-3.4	URFP-30-2	2 mL
Ultra Rainbow Fluorescent, 10^{7}/mL	3.0-3.4	URFP-30-20	20 mL
Ultra Rainbow Fluorescent, 10^{6}/mL, Ready-to-Use	3.0-3.4	URFP01-30-2K	2x15mL
Ultra Rainbow Fluorescent, 10^{6}/mL, Ready-to-Use	3.0-3.4	URFP01-30-10K	10x3mL
Ultra Rainbow Fluorescent, 10^{7}/mL	3.5-3.9	URFP-38-2	2 mL
Ultra Rainbow Fluorescent, Mid-Range Intensity, 10^{7}/mL	3.5-3.9	URFP-38-5A	5 mL
Ultra Rainbow Fluorescent, 10^{7}/mL	8.1-12.0	URFP-100-2	2 mL
Ultra Rainbow Fluorescent, $5x10^{6}$/mL	13.0-17.9	URFP-150-2	2 mL
Ultra Rainbow Fluorescent, 1% w/v	18.0-24.9	URFP-200-5	5 mL
Ultra Rainbow Fluorescent, 1% w/v	25.0-35.0	URFP-300-5	5 mL

Figure 52 Histograms of the Ultra Rainbow Fluorescent Particles (Cat. No. URFP-30-2, Lot No. AA02) on a Beckman Coulter Cyan™ ADP. NOTE: %CV is dependent on the flow rate, concentration, and the instrument used to evaluate the Ultra Rainbow Fluorescent Particles.



Spherotech Cat. No. URFP-30-2 Instructions for use:

A. Preparation of Particles

1. Vortex the particles vigorously
2. Add 2 to 4 drops of particles to 1mL of sheath fluid or DI water. The inclusion of a small amount of detergent (~0.01%) in the dilution buffer will increase the percentage of the singlet population.

B. Daily Alignment

To determine the optical alignment of the system perform the following:

1. Set a live gate for the singlet population on the FSC vs SSC histogram to exclude aggregates
2. Adjust the Gain and High voltage so that the mean channel number of the peak is in a predetermined position on each histogram of interest. The histograms on the previous page can be used as a guide.
3. Collect 5000 events inside the gate
4. Record the % CV and High Voltage for all fluorescence channels of interest.
5. Use a computer program such as Excel to generate the Levy Jennings graphs.

NOTE: If the values on any parameter exceed those of the day-to-day average or preset values, which are determined by at least one months worth of data, additional calibration or alignment procedures should be performed according to the instrument operation manual.

Figure 53 Levy Jennings Graph for the Voltage Setting used to place the URFP-30-2 at the 128 Channel Number on a Beckman Coulter CyAn™ ADP.

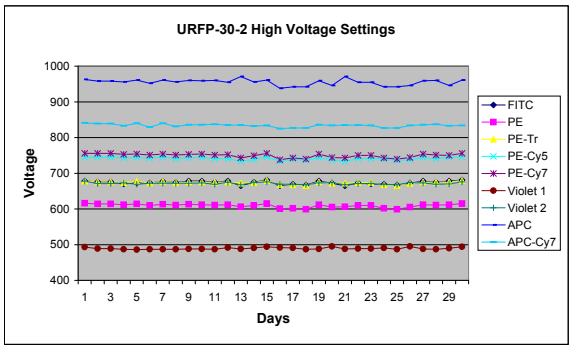
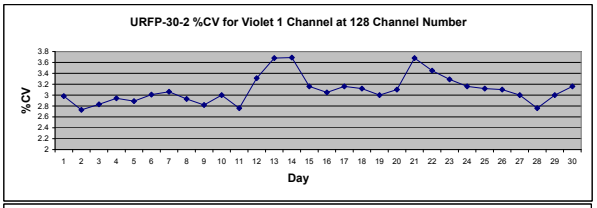
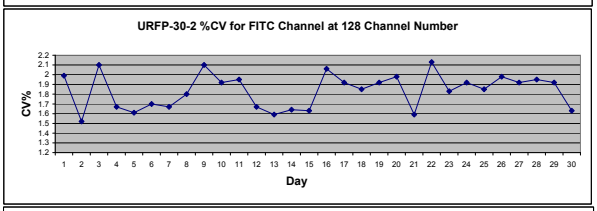
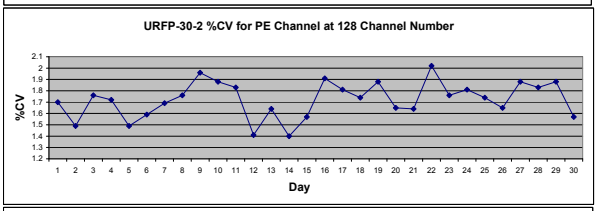
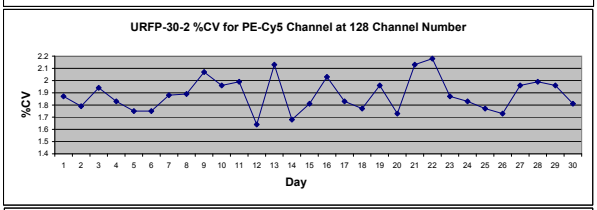


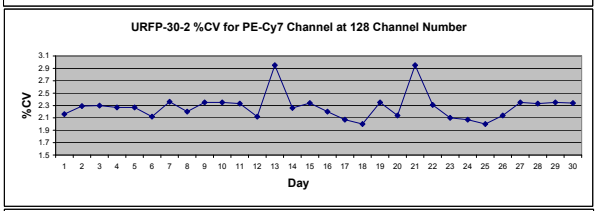
Figure 54 Levy Jennings Graphs for the %CV of the URFP-30-2 at the 128 Channel Number on a Beckman Coulter CyAn™ ADP.

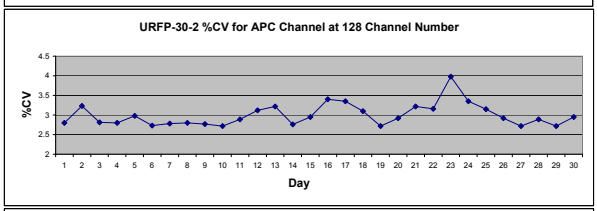












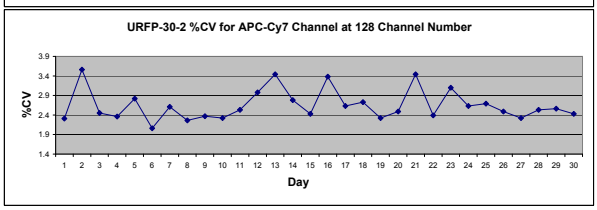
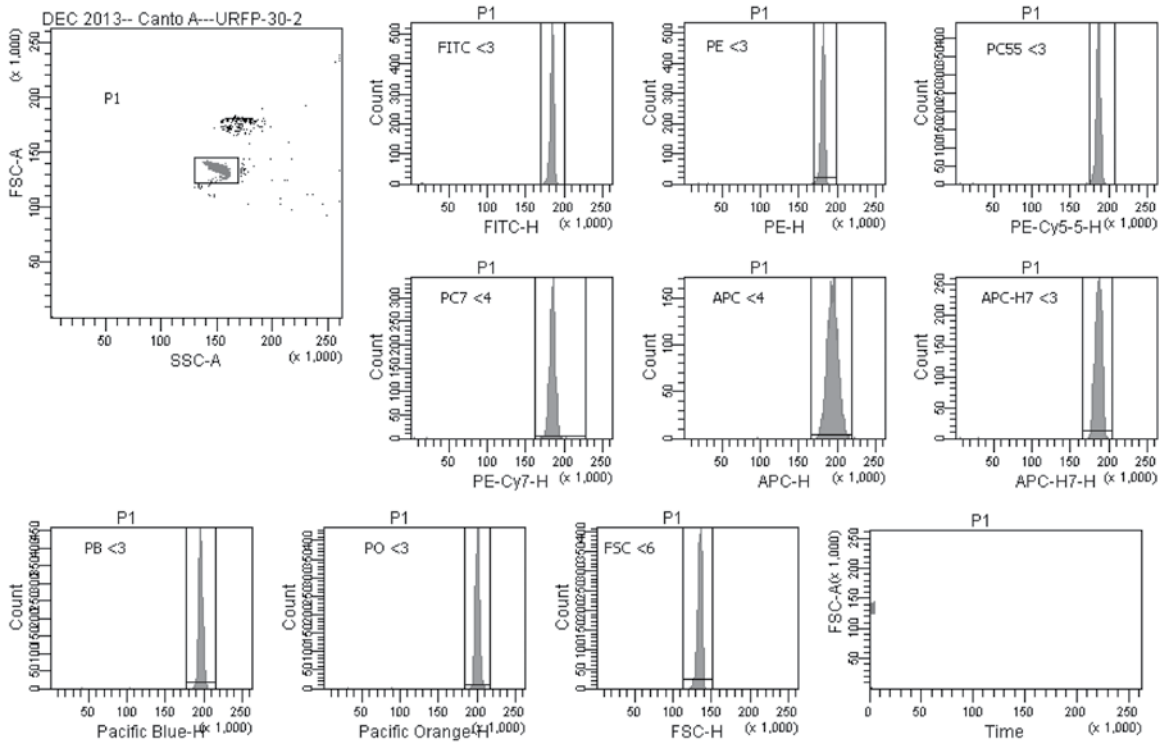


Figure 55 Histograms of the Ultra Rainbow Fluorescent Particles (Cat. No. URFP-30-2, Lot No. AE02) on a BD Canto™ II.



Population	FITC-H Mean	FITC-H %CV
FITC <3	184,254	1.3

Population	Pacific Blue-H Mean	Pacific Blue-H %CV
PB <3	196,026	1.3

Population	PE-H Mean	PE-H %CV
PE <3	181,156	1.3

Population	Pacific Orange-H Mean	Pacific Orange-H %CV
PO <3	200,938	1.3

Population	PE-Cy5-5-H Mean	PE-Cy5-5-H %CV
PC55 <3	186,116	1.4

Population	FSC-H Mean	FSC-H %CV
FSC <6	134,238	2.1

Population	PE-Cy7-H Mean	PE-Cy7-H %CV
PC7 <4	184,672	1.9

Population	APC-H Mean	APC-H %CV
APC <4	194,079	3.5

Population	APC-H7-H Mean	APC-H7-H %CV
APC-H7 <3	186,429	2.1

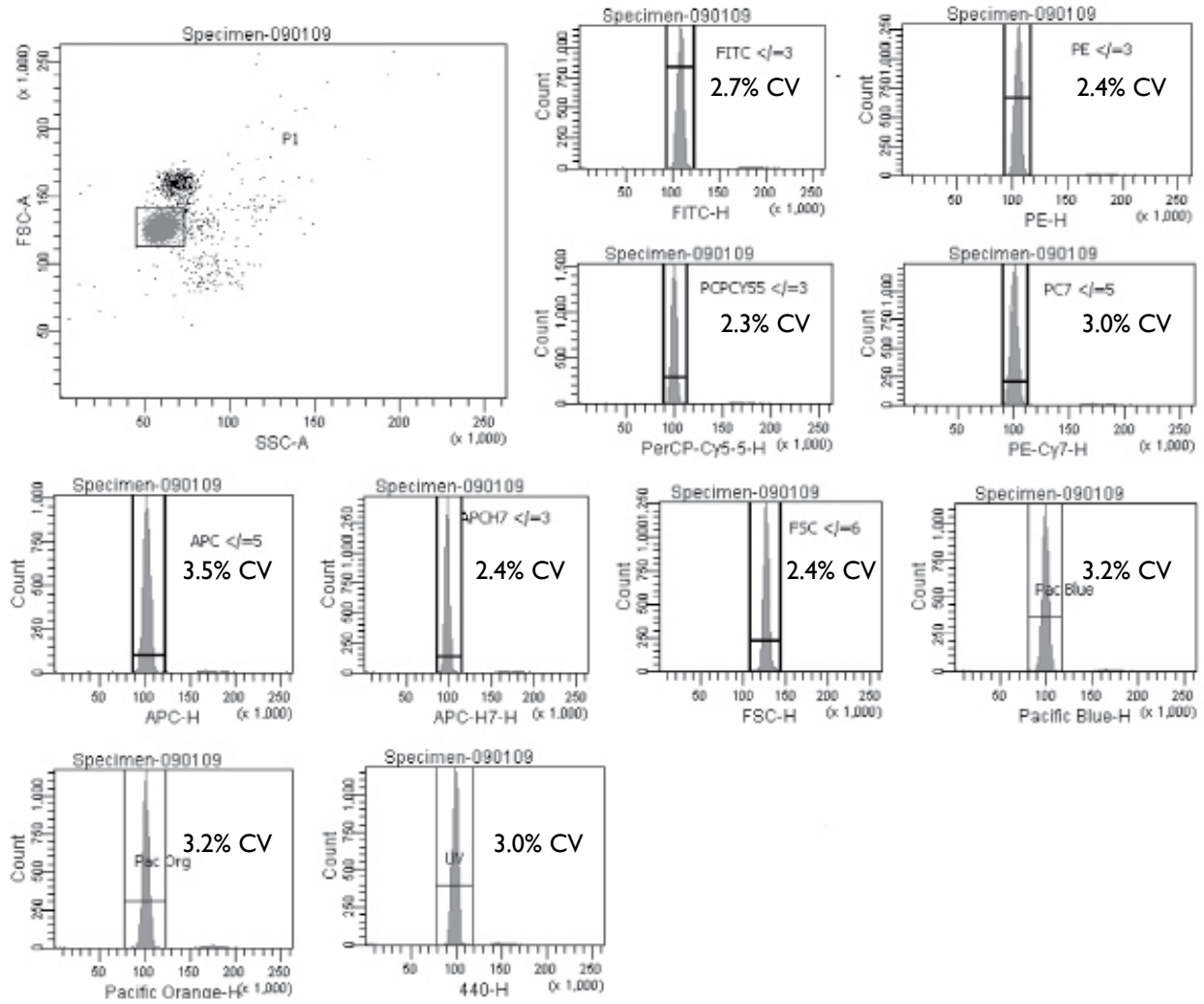
Tube: URFP-30-2

Population	#Events	%Parent	%Total
All Events	3,000	####	100.0
P1	2,729	91.0	91.0
FITC <3	2,727	99.9	90.9
PE <3	2,725	99.9	90.8
PC7 <4	2,727	99.9	90.9
APC <4	2,727	99.9	90.9
APC-H7 <3	2,727	99.9	90.9
FSC <6	2,729	100.0	91.0
PC55 <3	2,726	99.9	90.9
PB <3	2,727	99.9	90.9
PO <3	2,727	99.9	90.9

* Data provided by Laura Marszalek, Northwestern Memorial Hospital.

To see more information on Spherotech beads for flow cytometry calibration and standardization go to: ***www.Spherotech.com/tech.htm***

Figure 56 Histograms of the Ultra Rainbow Fluorescent Particles (Cat. No. URFP-30-2, Lot No. AA02) on a BD LSR™ II.



* Data provided by Laura Marszalek, Northwestern Memorial Hospital.

Figure 57 Histograms of the Ultra Rainbow Fluorescent Particles (Cat. No. URFP-38-2, Lot No. AB01) on a Beckman Coulter Cyan™ ADP.

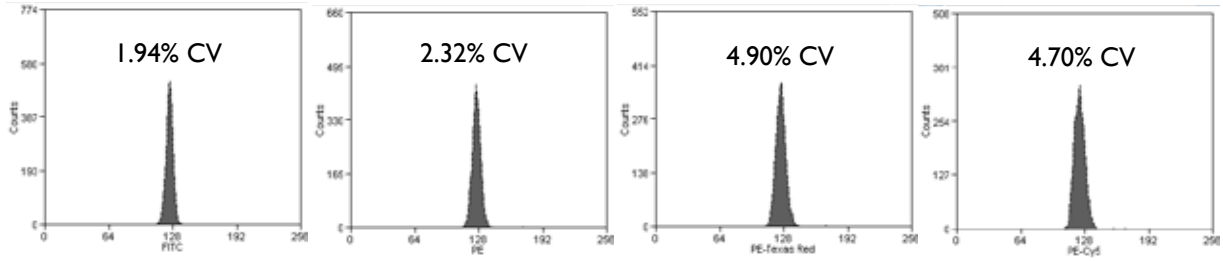


Figure 58 Histograms of the Ultra Rainbow Fluorescent Particles (Cat. No. URFP-100-2, Lot No. AF01) on a Beckman Coulter Cyan™ ADP.

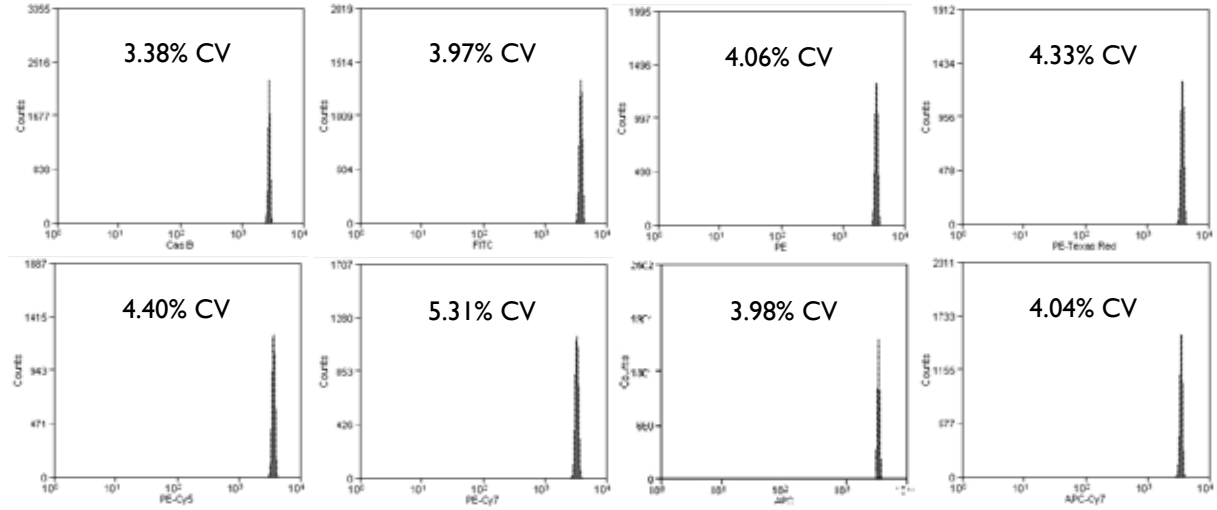


Figure 59 Histograms of the Ultra Rainbow Fluorescent Particles (Cat. No. URFP-30-2, Lot No. AG03) on a EMD Millipore Guava easyCyte™ 12.

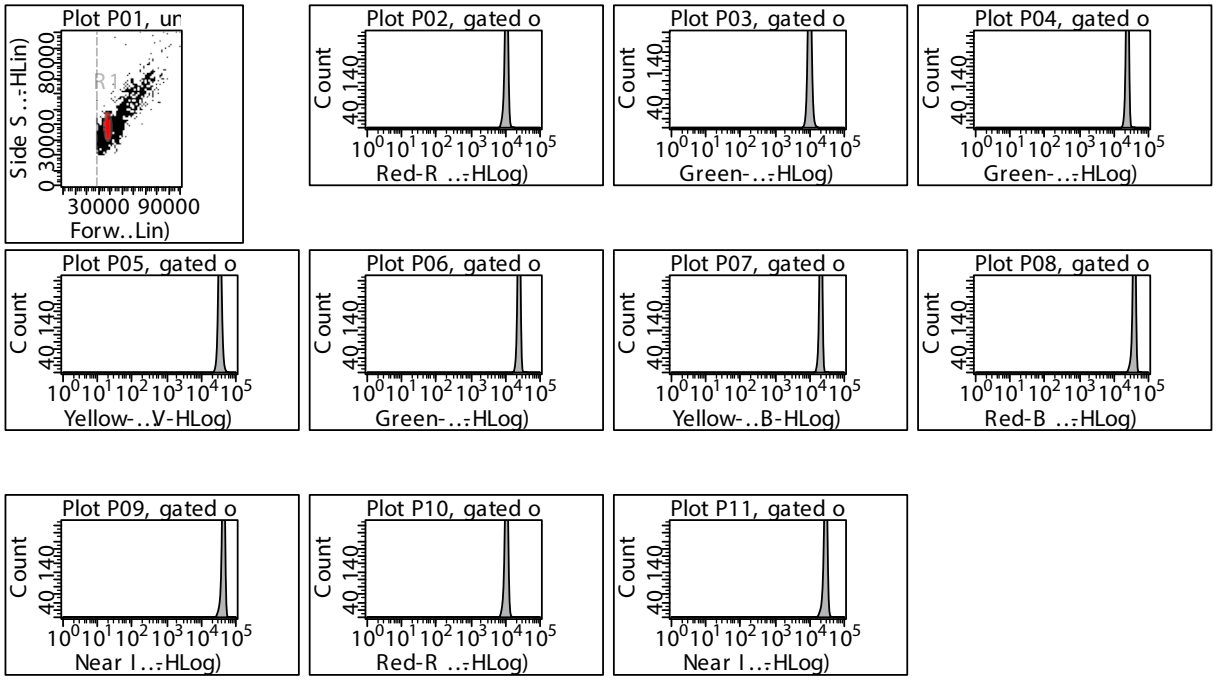


Figure 60 Histograms of the Ultra Rainbow Fluorescent Particles (Catalog No. URFP-30-2, Lot No. AG03) on a BD Bioscience LSRFortessa™ X-20 are shown below.

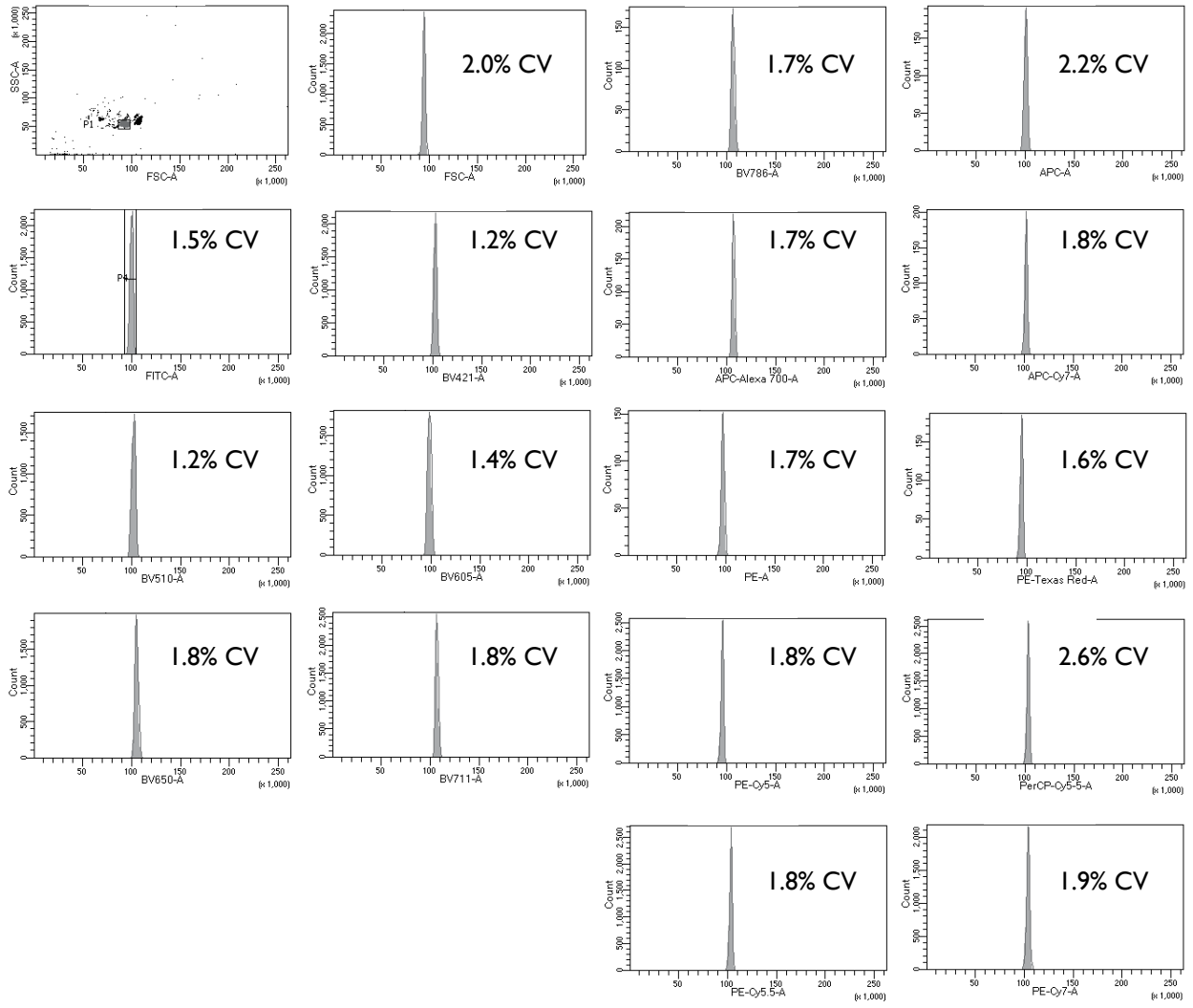
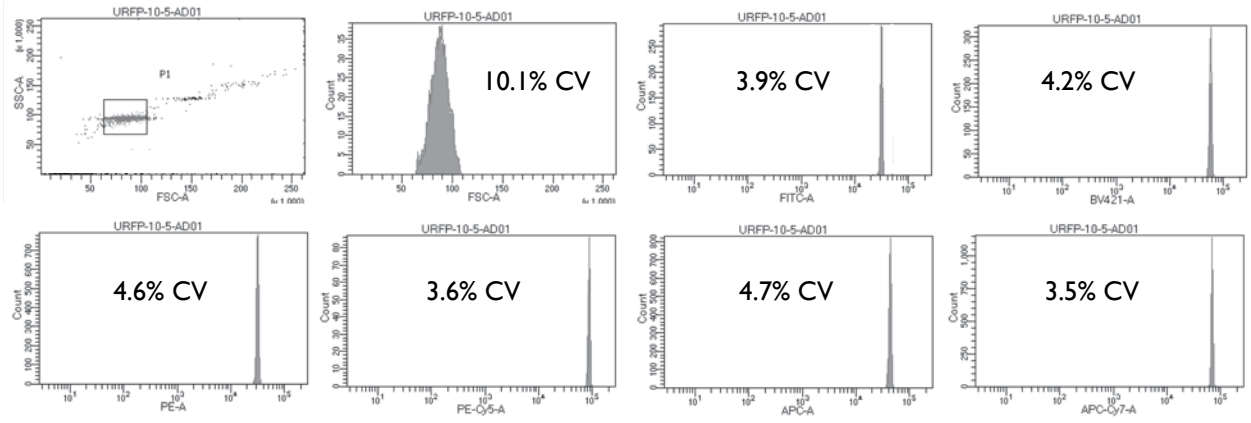


Figure 61 Histograms of the Ultra Rainbow Fluorescent Particles (Catalog No. URFP-10-5, Lot No. AG03, 1.06 micron) on a BD Bioscience LSRFortessa™ X-20 are shown below.below.

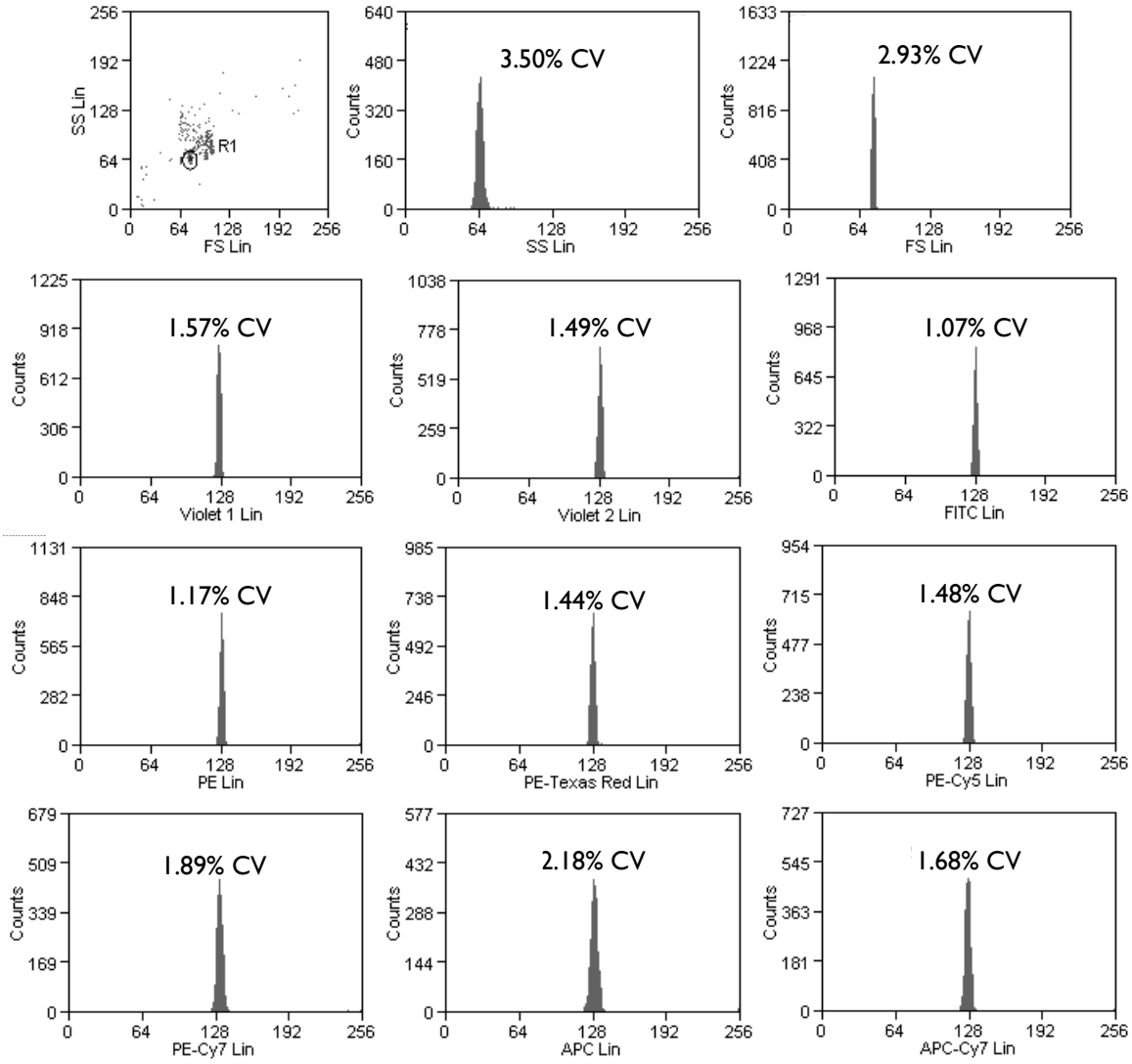


SPHERO™ Ultra Rainbow Fluorescent Particles for Beckman Coulter Cytometers

- Consists of a single peak for performance verification of Beckman Coulter flow cytometers
- Determines cleanliness of the flow cell and fluidics

Particle Type and Surface	Size, µm	Catalog No.	Unit
Calibration Beads for MoFlo™ Astrios™, 10^7/mL	3.0-3.4	B27034	2 mL
Calibration Beads for MoFlo™, MoFlo™ XDP, and CyAn™ ADP, 10^7/mL	3.0-3.4	B28479	2 mL
AccuCount Ultra Rainbow Fluorescent Particles (Calibration Beads for Aquios CL™, 10^6/mL)	3.8 (+/-0.3)	ACURFP-38-5	5 mL
AccuCount Ultra Rainbow Fluorescent Particles (Calibration Beads for Aquios CL™, 10^6/mL)	3.8 (+/-0.3)	ACURFP-38-15	15 mL

Figure 62 Histograms of the Calibration Beads for MoFlo™, MoFlo™ XDP, and CyAn™ ADP (Cat. No. B28479, Lot No. BAE01) on a Beckman Coulter Cyan™ ADP.



SPHERO™ Rainbow Fluorescent Particles

The Rainbow Fluorescent Particles are similar to Rainbow Calibration Particles except that these represent uniform size particles with a single intensity. The Rainbow Fluorescent Particles are usually the brightest peak of the corresponding Rainbow Calibration Particles with the exception of RFP-50-5, RFP-70-2, RFP-100-2 and RFP-30-5A. The RFP-30-5A has the fluorescence intensity similar to stained cells in all channels. Since these particles contain a single peak with very small fluorescence and size CV, they are very useful in the alignment of the optical system of the flow cytometer in all channels.

Particle Type and Surface	Size, µm	Catalog No.	Unit
Rainbow Fluorescent, 10^7/mL (Intensity similar to brightest peak in RCP-20-5)	1.8-2.2	RFP-20-5	5 mL
Rainbow Fluorescent, 10^7/mL (Intensity similar to brightest peak in RCP-30-5)	3.0-3.4	RFP-30-5	5 mL
Rainbow Fluorescent, 10^7/mL (Intensity similar to mid range FL1 fluorescence in RCP-30-5)	3.0-3.4	RFP-30-5A	5 mL
Rainbow Fluorescent, 10^7/mL (Intensity similar to brightest peak in RCP-35-5)	3.5-4.0	RFP-35-5	5 mL
Rainbow Fluorescent, 10^7/mL	5.0-5.9	RFP-50-5	5 mL
Rainbow Fluorescent, 10^7/mL (Intensity similar to brightest peak in RCP-60-5)	6.0-6.4	RFP-60-5	5 mL
Rainbow Fluorescent, 10^7/mL	6.5-8.0	RFP-70-5	5 mL
Rainbow Fluorescent, 10^7/mL	8.1-12.0	RFP-100-2	2 mL

Figure 63 Histograms of the Rainbow Fluorescent Particles (Cat. No. RFP-30-5, Lot No. AF02).

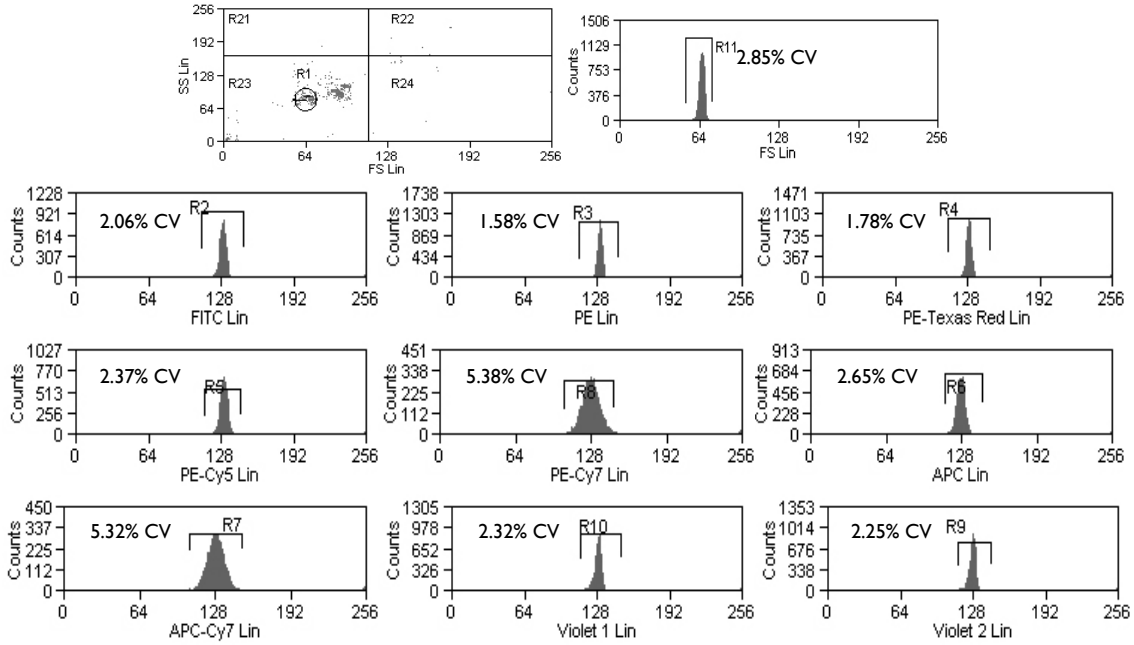


Figure 64 Histograms of the Rainbow Fluorescent Particles, Mid-Range (Cat. No. RFP-30-5A, Lot No. AF02).

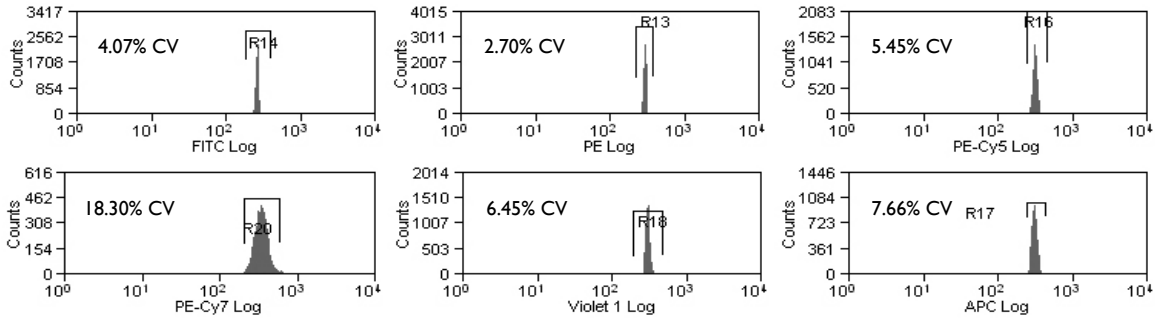


Figure 65 Histograms showing fluorescence intensities of Rainbow Fluorescent Particles (Catalog No. RFP-100-2, Lot No. AE01) on a BD Bioscience LSRFortessa™ X-20 are shown below.

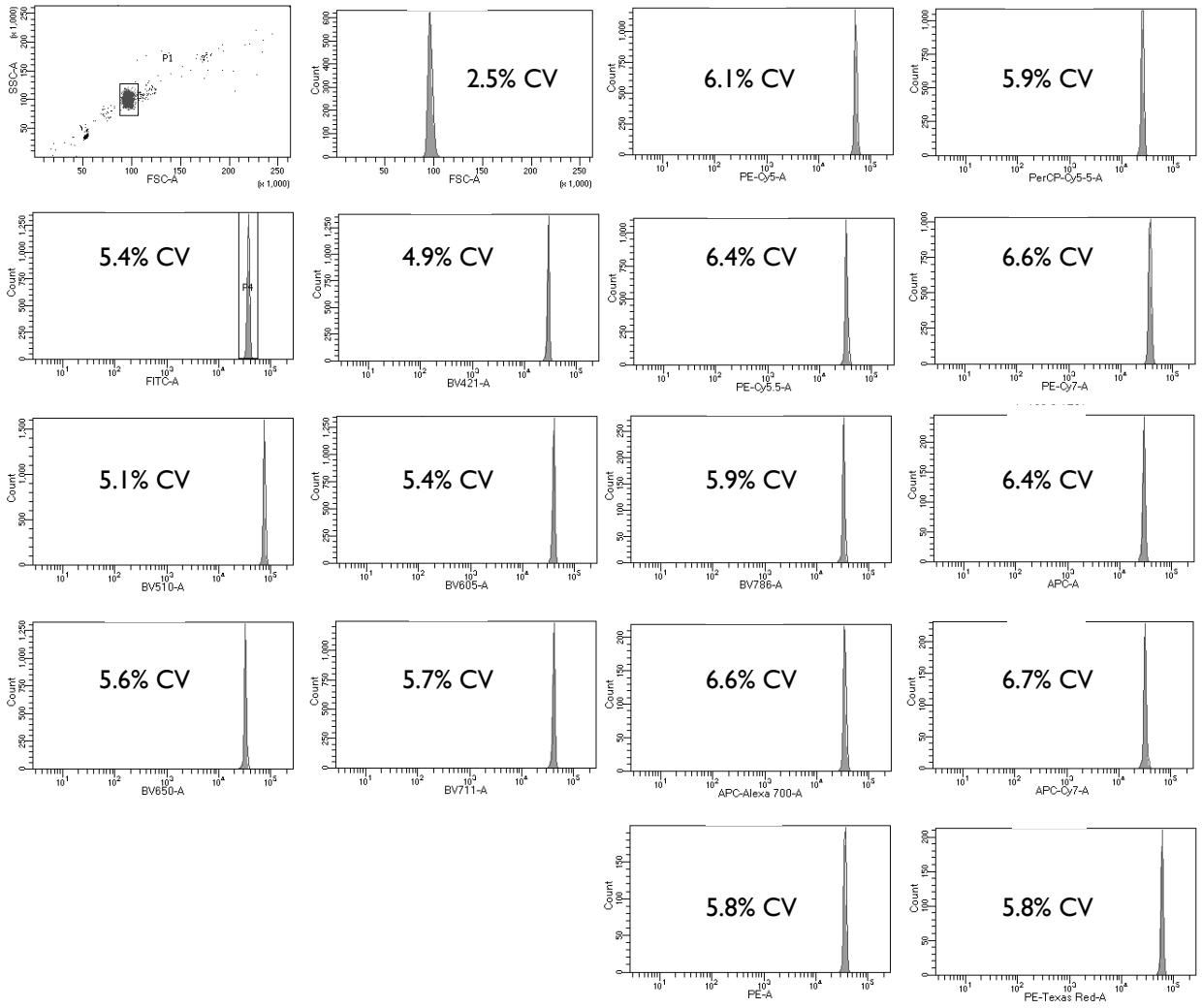
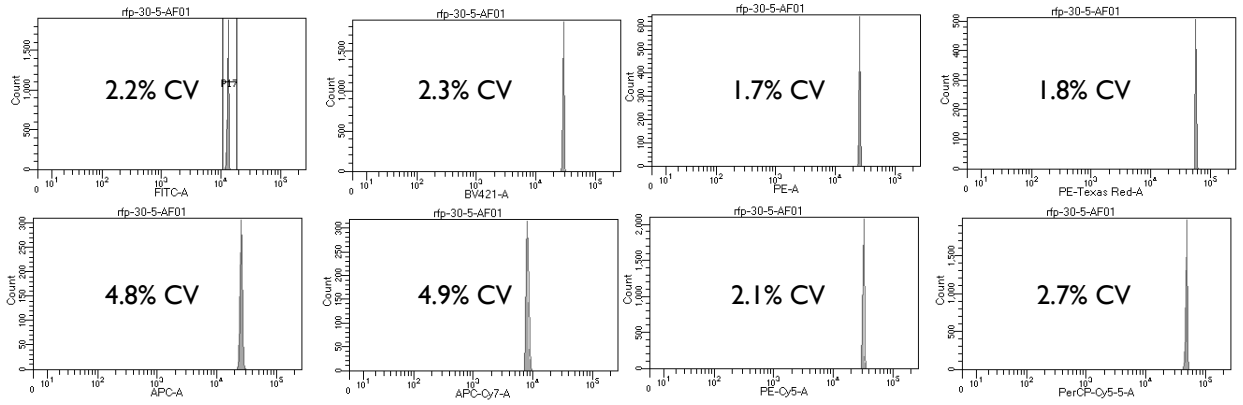


Figure 66 Histograms showing fluorescence intensities of Rainbow Fluorescent Particles (Catalog No. RFP-30-2, Lot No. AF02) on a BD Bioscience LSRFortessa™ X-20 are shown below.



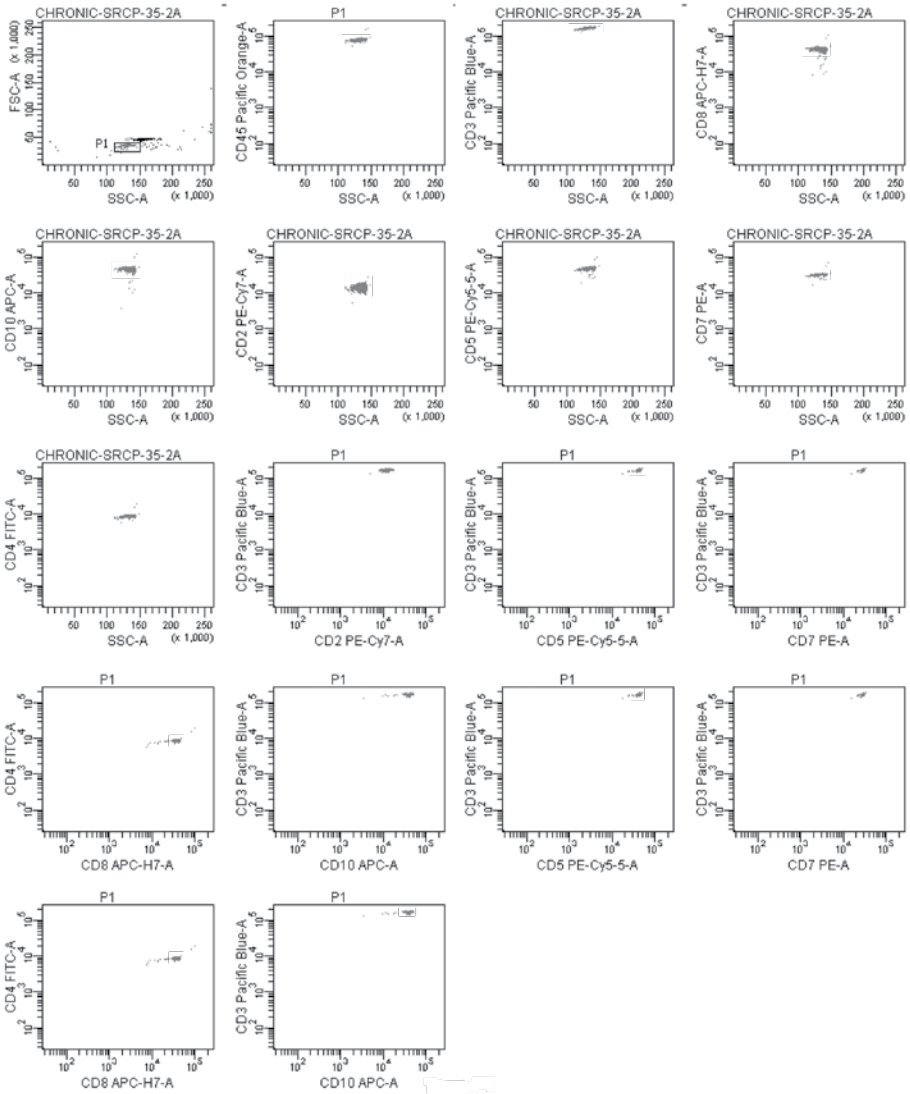
SPHERO™ Supra Rainbow Midrange Fluorescent Particles

- Consists of a single peak which has an intensity similar to real samples
- Contains a single peak with very low fluorescence and size CV
- Fluorescent in UV, FITC, PE, PE-TR, PE-Cy5, PE-Cy7, APC, APC-Cy7, and IR
- Measures the coefficients of variation (CVs), and target channels using experimental setting.

Particle Type and Surface	Size, µm	Catalog No.	Unit
Supra Rainbow Midrange Fluorescent, 10^7/mL	3.0-3.59	SRCP-35-2A	2 mL

The Supra Rainbow Midrange Fluorescent Particles (SRCPs) have an intensity close to that of cellular samples with excellect CVs. As a result, once the optimal voltages for a particular experiment are determined, the setting can be captured as target channels based on the mean fluorescence intensity of the SRCPss. This allows creation of Levy-Jennings plots of the voltages to get the beads to a specific target channel number. The target channel numbers are more robust to instrument changes than the voltages themselves. As a result, changes in the instrument are easier to detect.

Figure 67 Histograms of the Supra Rainbow Midrange Fluorescent Particles (Cat. No. SRCP-35-2A) using the setting of cellular samples.

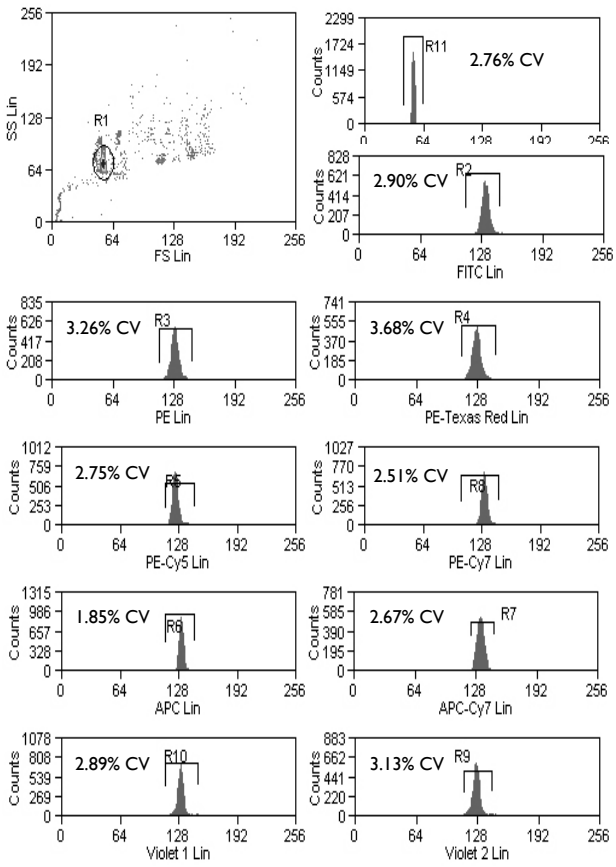


SPHERO™ Rainbow Alignment Particles

- Consists of a prediluted, single peak for optical alignment of any flow cytometer in any channel
- Contains a single peak with very low fluorescence and size CV
- Fluorescent in UV, FITC, PE, PE-TR, PE-Cy5, PE-Cy7, APC, and APC-Cy7 channels
- Measures the coefficients of variation (CVs), peak channels, and histogram distribution of flow cytometers.

Particle Type and Surface	Size, µm	Catalog No.	Unit
Rainbow Fluorescent Alignment Particles, 1.2x10^6/mL	3.5-3.9	RAP-38-5	5 mL

Figure 68 Histograms of the Rainbow Alignment Particles (Cat. No. RAP-38-5, Lot No. AD01) on a Beckman Coulter Cyan™ ADP.

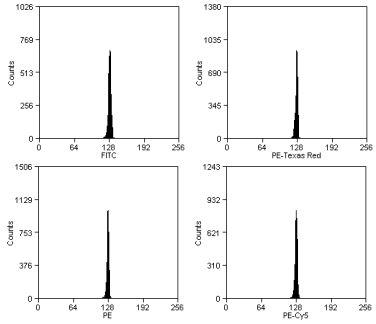


SPHERO™ Fluorescent Alignment Particles

- Consists of a single peak for optical alignment of any flow cytometer
- Contains a single peak with very low fluorescence and size CV
- Fluorescent in FITC, PE, PE-TR, and PE-Cy5 channels
- Measures the coefficients of variation (CVs), peak channels, and histogram distribution of flow cytometers.

Particle Type and Surface	Size, µm	Catalog No.	Unit
Fluorescent Alignment Particles, 10^8/mL	3.0-3.4	FAP-3056-5	5 mL

Figure 69 Histograms of the Fluorescent Alignment Particles (Cat. No. FAP-3056-5, Lot No. AG01) on a Beckman Coulter Cyan™ ADP.



SPHERO™ Rainbow Calibration and Rainbow QC Kits

The Rainbow Calibration Kit and Rainbow QC Kit are designed to simplify the routine calibration of flow cytometers.

SPHERO™ Rainbow QC Kit (Cat. No. RCK-3K)
Consists of one vial each of the following particles:
RCP-30-5A-1 (Peak 1), 3.0-3.4µm, 10^7/mL, 5mL
RCP-30-5A-4 (Peak 4), 3.0-3.4µm, 10^7/mL, 5mL
RCP-30-5A-8 (Peak 8), 3.0-3.4µm, 10^7/mL, 5mL

SPHERO™ Rainbow Calibration Kit (Cat. No. RQC-4K)
Consists of one vial each of the following particles:
BCP-60-2, 6.0-6.4µm, 10^7/mL, 2mL
RFP-60-2, 6.0-6.4µm, 10^7/mL, 2mL
RCP-30-2L (Peaks 1-4), 3.0-3.4µm, 10^7/mL, 2mL
RCP-30-2H (Peaks 5-8), 3.0-3.4µm, 10^7/mL, 2mL

Particle Type and Surface	Catalog No.	Unit
Rainbow Calibration Kit	RCK-3K	1 Kit
Rainbow QC Kit	RQC-4K	1 Kit

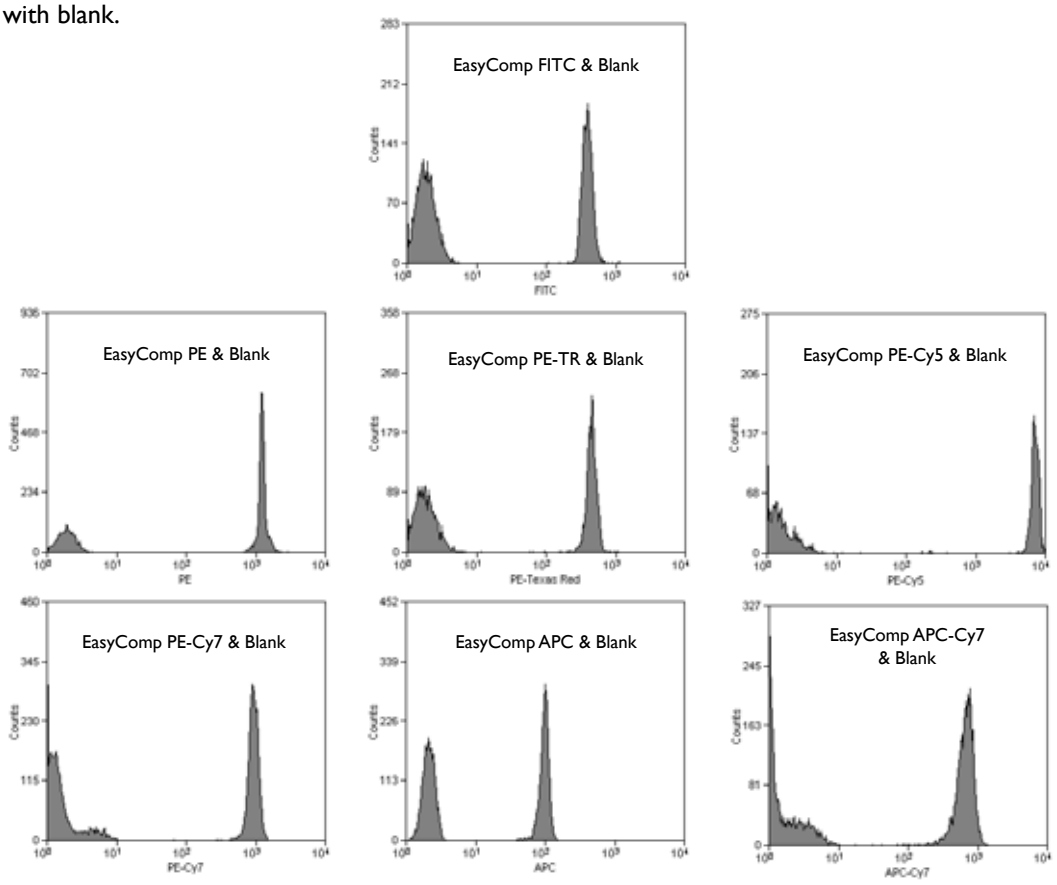
SPHERO™ EasyComp Fluorescent Particles

- Prepared by labeling the surface of polystyrene particles with commonly used fluorophores
- Provide spectral matching particles for setting compensation in a wide variety of channels for any flow cytometer
- Supplied as individual products or as kits for different flow cytometer models.

The EasyComp Fluorescent Particles are prepared by surface labeling polystyrene particles with commonly used fluorophores. These particles are used for setting compensation in a wide variety of channels for any flow cytometer. The EasyComp Fluorescent Particles are supplied as individual products or as kits for use in different models of flow cytometers. For more information on Spherotech beads for flow cytometry compensation go to *www.Spherotech.com/pdetail.htm* and select Flow Cytometry Compensation Particles.

Particle Type and Surface	Size, µm	Catalog No.	Unit
EasyComp Blank, 10^7/mL	3.0-3.4	ECFP-B	5 mL
EasyComp FITC, 10^7/mL	3.0-3.4	ECFP-F1	1 mL
EasyComp PE, 10^7/mL	3.0-3.4	ECFP-F2	1 mL
EasyComp PE-TR, 10^7/mL	3.0-3.4	ECFP-F3	1 mL
EasyComp PE-Cy5, 10^7/mL	3.0-3.4	ECFP-F4	1 mL
EasyComp PE-Cy7, 10^7/mL	3.0-3.4	ECFP-F5	1 mL
EasyComp APC, 10^7/mL	3.0-3.4	ECFP-F6	1 mL
EasyComp APC-Cy7, 10^7/mL	3.0-3.4	ECFP-F7	1 mL
EasyComp GFP, 10^7/mL	3.0-3.4	ECFP-F9	1 mL
EasyComp Kit, (Blank, FITC, PE & PE-Cy5), 4 vials, 10^7/mL	3.0-3.4	ECFP-K1	1x5 mL & 3x1 mL
EasyComp Kit, (Blank, FITC, PE, PE-Cy5 & APC), 5 vials, 10^7/mL	3.0-3.4	ECFP-K2	1x5 mL & 4x1 mL
EasyComp Kit, (Blank, FITC, PE, PE-TR & PE-Cy5), 5 vials, 10^7/mL	3.0-3.4	ECFP-K3	1x5 mL & 4x1 mL
EasyComp Kit, (Blank, FITC, PE, PE-TR, PE-Cy5, PE-Cy7, APC & APC-Cy7), 8 vials, 10^7/mL	3.0-3.4	ECFP-K4	1x5 mL & 7x1 mL
EasyComp Fluorescent Particle Kit (Blank, FITC, PE, PE-TR, PE-Cy5, APC & APC-Cy7), 7 vials, 10^7/mL	3.0-3.4	ECFP-K5	1x5 mL & 6x1 mL

Figure 70 Histograms of the FITC, PE, PE-TR, PE-Cy5, PE-Cy7, APC, and APC-CY7 Easy Comp Compensation Particles with blank.



SPHERO™ COMPtrol Particles

- Coated with Goat anti-Mouse Ig (H&L) to bind to fluorochrome-conjugated monoclonal antibodies used in cell staining
- Provides a method for the quality control of the fluorochrome-conjugated monoclonal antibodies
- Aids in setting proper compensation to reduce cross-talk between flow cytometer channels

The COMPtrol Particles are coated with Goat anti-Mouse Ig (H&L). They bind to fluorochrome-conjugated monoclonal mouse antibodies used during cell staining. These particles provide a method for the quality control of these conjugates. They are also used to aid in setting proper compensation to reduce the cross-talk between the channels of multicolor flow cytometers.

Particle Type and Surface	Size, µm	Catalog No.	Unit
COMPtrol Kit, Goat anti-Mouse Ig (H&L) Coated Particles, 2 populations (Negative & High), $1x10^7$/mL	0.7-0.9	CMIgP-08-2K	2x5mL
COMPtrol Kit, Goat anti-Mouse Ig (H&L) Coated Particles, 2 populations (Negative & High), $1x10^7$/mL	3.0-3.4	CMIgP-30-2K	2x5mL
COMPtrol Kit, Goat anti-Mouse Ig (H&L) Coated Particles, 3 populations (Negative, Low, & High), $2.5x10^6$/mL	5.0-5.9	CMIgP-50-3K	3x5mL
COMPtrol Kit, Goat anti-Mouse Ig (H&L) Coated Particles, 3 populations (Negative, Low, & High), $2.5x10^6$/mL	7.0-7.9	CMIgP-70-3K	3x5mL

Figure 71 Histograms of the 3 micron COMPtrol Particles (Cat. No. CMIgP-30-2K) stained with mouse monoclonal fluorescent conjugate.

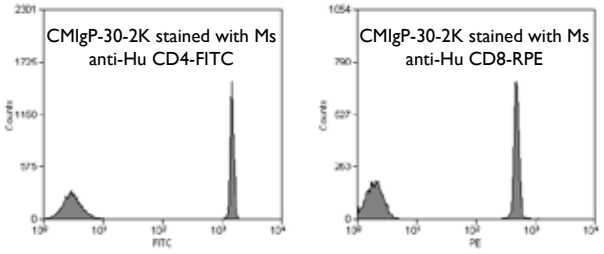
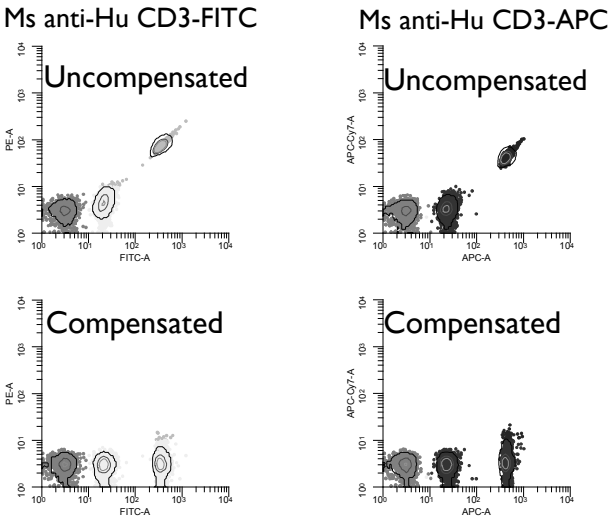


Figure 72 Histograms of the 5 micron COMPtrol Particles (Cat. No. CMIgP-50-3K) stained with mouse monoclonal fluorescent conjugate.

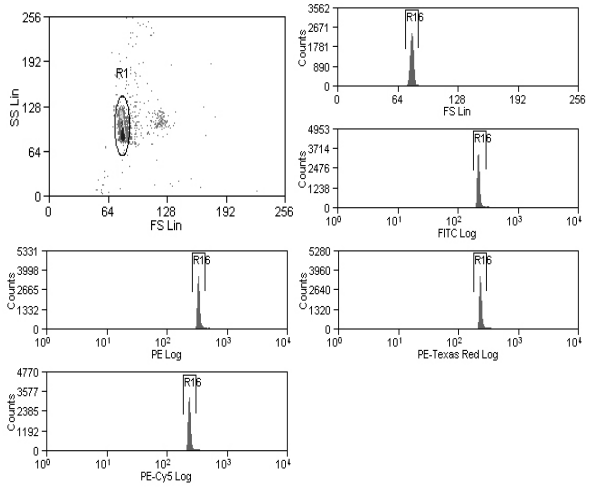


SPHERO™ AccuCount Particles

- Contains particles with a known number of particles per mL
- Easy to use and cost effective
- Available in several sizes to accommodate the target cell size to be counted
- Provided as blank, fluorescent, Rainbow, or Ultra Rainbow particles that can be used in multiple fluorescent channels of flow cytometers.

The SPHERO™ AccuCount Particles are designed to be used as reference particles with known number of particles per mL for counting the absolute cell number by flow cytometry. The SPHERO™ AccuCount Particles are very easy to use and are cost effective. The AccuCount Fluorescent Particles are fluorescent in FITC, PE and PE-Cy5 channel. Both AccuCount Fluorescent and AccuCount Blank (nonfluorescent) Particles are available in various particle sizes to accommodate the size of the cells to be counted. In addition, Spherotech also manufactures the AccuCount Rainbow Fluorescent Particles and AccuCount Ultra Rainbow Fluorescent Particles for detection in more fluorescent channels.

Figure 73 Histograms of Cat. No. ACFP-100-3 (AccuCount Fluorescent Particles, 10^6/mL, 10.2 µm, 3 mL) on a Stratedigm S1400.



To see more information on Spherotech Absolute Counting beads for flow cytometry go to: ***www.Spherotech.com/tech.htm***

Particle Type and Surface	Size, µm	Catalog No.	Unit
AccuCount Blank, 10^6/mL	2.0-2.4	ACBP-20-10	10 mL
AccuCount Blank, 10^6/mL	3.0-3.9	ACBP-30-10	10 mL
AccuCount Blank, 10^6/mL	5.0-5.9	ACBP-50-10	10 mL
AccuCount Blank, 10^6/mL	7.0-7.9	ACBP-70-10	10 mL
AccuCount Blank, 10^6/mL	8.0-12.9	ACBP-100-10	10 mL
AccuCount Blank, 10^6/mL	13.0-17.9	ACBP-150-10	10 mL
AccuCount Fluorescent, 10^6/mL	5.0-5.9	ACFP-50-5	5 mL
AccuCount Fluorescent, 10^6/mL	7.0-7.9	ACFP-70-5	5 mL
AccuCount Fluorescent, 10^6/mL	7.0-7.9	ACFP-70-10	10 mL
AccuCount Fluorescent, 10^6/mL	8.0-12.9	ACFP-100-3	3 mL
AccuCount Rainbow Fluorescent, Low Intensity, 10^6/mL	8.0-12.9	ACRFL-100-3	3 mL
AccuCount Rainbow Fluorescent, 10^6/mL	8.0-12.9	ACRFP-100-3	3 mL
AccuCount Ultra Rainbow Fluorescent, 10^6/mL	5.0-5.9	ACURFP-50-10	10 mL
AccuCount Fluorescent Particle Kit. Contains 4 bottles: 0.5x10^6mL, 1.0x10^6/mL, 0.5x10^7mL, & 1.0x10^7/mL	5.0-5.9	ACFP-50-4K	4x1 mL

Figure 74 Histograms of 5 micron fluorescent particles spiked with Cat. No. ACFP-100-3.

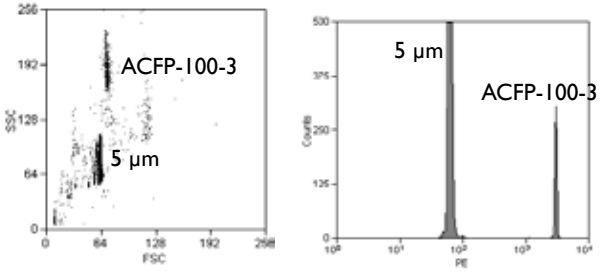
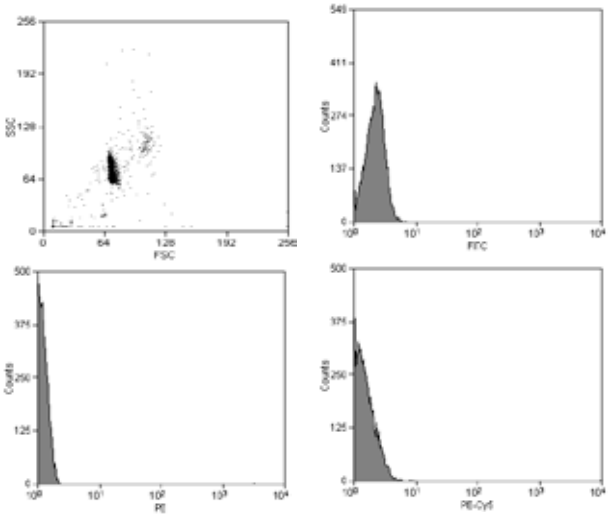


Figure 75 Histograms of Cat. No. ACBP-100-3 (AccuCount Blank Particles, 10^6/mL, 10.2 µm, 10 mL).



SPHERO™ Drop Delay Calibration Particles

- Aids in the determination of the drop delay value
- Increases the accuracy and productivity while sorting
- Consists of 2 mL at $1x10^8$ particles/mL of a single population of fluorescent particles
- Contains a mixture of fluorophores which allows detection in multiple channel flow cytometers.

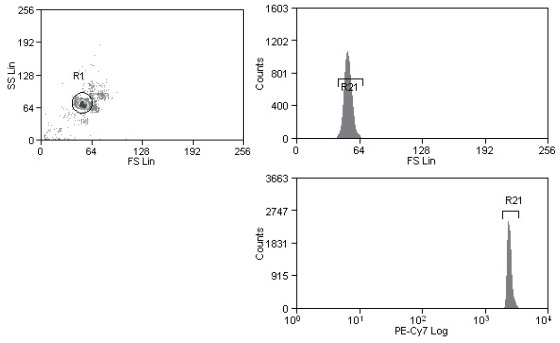
The SPHERO™ Drop Delay Calibration Particles are fluorescent particles to aid in the determination of the drop delay value for flow cytometer sorters with the appropriate attachment. As a result of using the Drop Delay Calibration Particles and the appropriate attachment, the accuracy and productivity during sorting is enhanced.

Figure 76 Initial Drop Delay Profile with Spherotech Drop Delay Calibration Particles.

Figure 77 Optimized Drop Delay Profile with Spherotech Drop Delay Calibration Particles.

Particle Type and Surface	Size, µm	Catalog No.	Unit
Drop Delay Calibration, 10^8/mL	6.0-8.0	DDCP-70-2	2 mL
Drop Delay Calibration, 10^8/mL	6.0-8.0	DDCP-70-20	20 mL

Figure 78 Histograms of Drop Delay Calibration Particles, Cat. No. DDCP-70-2.



Spherotech Inc.
Specializing in Microparticle Technology

OEM Capabilities
- Custom Microparticle Synthesis
- Custom Microparticle Coating
- Contract Research
- Feasibility Assessment
- Customized Packaging
- Inventory Management
- Bulk Formulations

Technical Support
- Assay Optimization
- Formulation Development
- Application Support

Particles manufactured by Spherotech are utilized in:
- Fluorescence Immunoassay
- Enzyme Immunoassay (EIA)
- Fluorescence Microscopy
- Confocal Fluorescence Microscopy
- Flow Cytometry / Image Cytometry
- Magnetic Cell Separation
- Magnetic Particles EIA
- Microfluidics
- Nanotechnology
- Other Research and Industrial Applications.

Our loyal customers value Spherotech's agile manufacturing capabilities, custom OEM particle solutions, and value-add supply options. Our manufacturing facilities can accommodate multi-liter lot sizes of our entire microsphere offering.

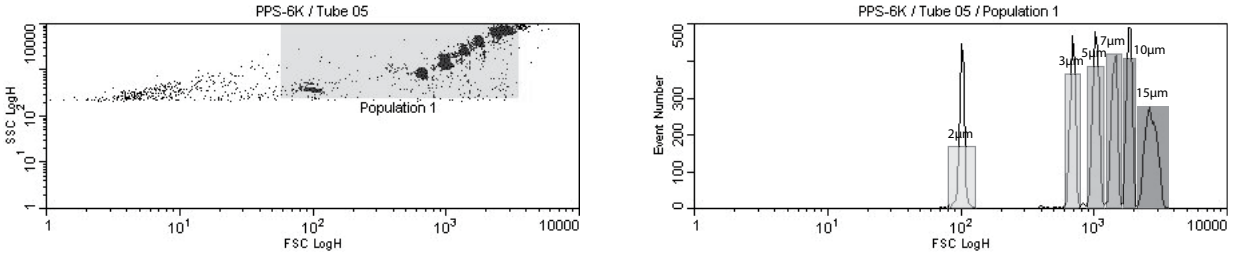
SPHERO™ Flow Cytometry Particle Size Standard Kit

The SPHERO™ Flow Cytometry Particle Size Standard Kit is designed to be a reliable size reference for flow cytometry. This kit consists of six different size particles with a known diameter. The diameter for each particle has been determined using a Scanning Electron Microscope and NIST traceable particles.

Using FSC signals of the flow cytometry, the size of cells can be estimated when compared to the SPHERO™ Flow Cytometry Particle Size Standards. When using this product, be aware that FSC signals are related to both size and refractive index.

Particle Type and Surface	Size, µm	Catalog No.	Unit
Particle Size Standard Kit, Flow Cytometry Grade, 2.5x10^6/mL	2.0-2.4, 3.0-3.4, 5.0-5.9, 7.0-7.9, 8.0-12.9, & 13.0-17.9	PPS-6K	6x5 mL

Figure 79 FSC Log Histograms of Cat. No. PPS-6K, Lot No. AG03 on a Stratedigm S1400.



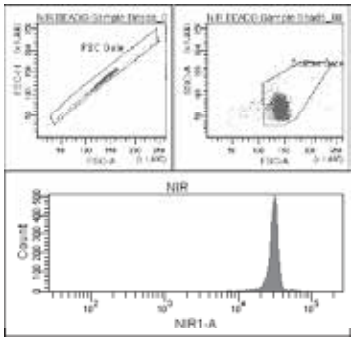
SPHERO™ Flow Cytometry Grade Fluorescent Particles

- Designed for flow cytometry applications
- Manufactured from high grade polystyrene particles
- Available in a variety of sizes and chemistries

Note: Many of the beads on pages 14 to 19 are also useful in flow cytometry applications.

Fluorescent Particles	Excitation	Emission
UltraBlue	635 or 785 nm	APC-Cy7 / IR
CyGreen	635 or 785 nm	APC-Cy7 / IR
Aqua Green	635 or 785 nm	APC-Cy7 / IR
Jade Green	635 or 785 nm	APC-Cy7 / IR

Figure 80 Histograms of Cat. No. CFH-5078-2 at 735nm Ex dected by a PMT with 840/30 nm BP.



* Data provided by David Haviland, Ph.D., University of Texas Health Science Center - Houston Center for Stem Cell Research - Flow Cytometry Laboratory

SPHERO™ Fluorescent IR Flow Cytometer Grade Particles

Particle Type and Surface	Size, µm	Catalog No.	Unit
Fluorescent, CyGreen, 10^7/mL	2.8-3.4	FP-3074-2	2 mL
Fluorescent, Jade Green, 10^7/mL	2.8-3.4	FP-3078-2	2 mL
Fluorescent, Aqua Green, 10^7/mL	3.0-3.4	FP-3079-2	2 mL
Fluorescent, CyGreen, 10^7/mL	5.0-5.9	FP-5074-2	2 mL
Fluorescent, Jade Green, 10^7/mL	5.0-5.9	FP-5078-2	2 mL
Fluorescent, CyGreen, Low Intensity, 10^7/mL	10.0-14.0	FL-10074-2	2 mL
Fluorescent, CyGreen, Mid Intensity, 10^7/mL	10.0-14.0	FP-10074-2	2 mL
Fluorescent, CyGreen, High Intensity, 10^7/mL	10.0-14.0	FH-10074-2	2 mL
Fluorescent, Jade Green, Low Intensity, 10^7/mL	10.0-14.0	FL-10078-2	2 mL
Fluorescent, Jade Green, Mid Intensity, 10^7/mL	10.0-14.0	FP-10078-2	2 mL
Fluorescent, Jade Green, High Intensity, 10^7/mL	10.0-14.0	FH-10078-2	2 mL
Fluorescent, Aqua Green, 10^7/mL	10.0-14.0	FP-10079-2	2 mL

Figure 81 Spectra of CyGreen, Jade Green and Aqua Green fluorophores at 640 nm excitation.

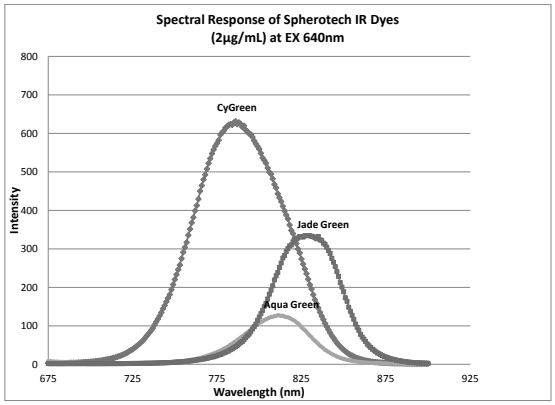
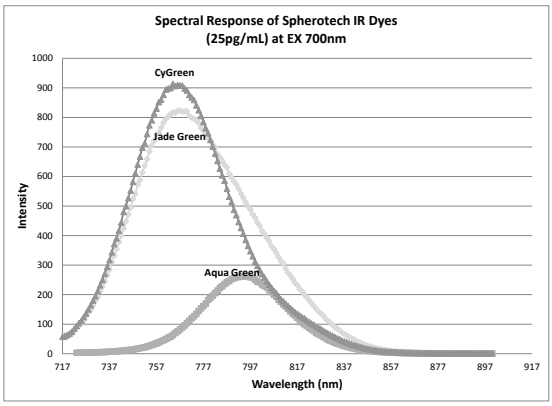
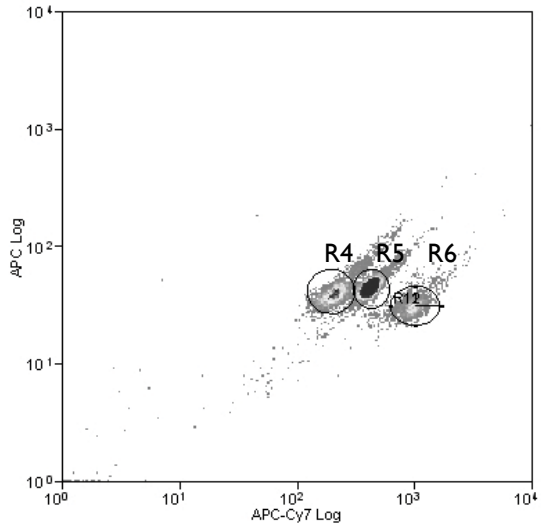


Figure 82 Fluorescence data for CyGreen, Jade Green, and Aqua Green. (a) Spectra of CyGreen, Jade Green and Aqua Green fluorophores at 700 nm excitation. (b) Histograms of Cat. No. CFP-5074-2 (R4), CFP-5078-2 (R5) & CFP-5079-2 (R6) at 635nm Ex dected in the ACP-Cy7 channel of a Beckman Coulter Cyan ADP

(A)



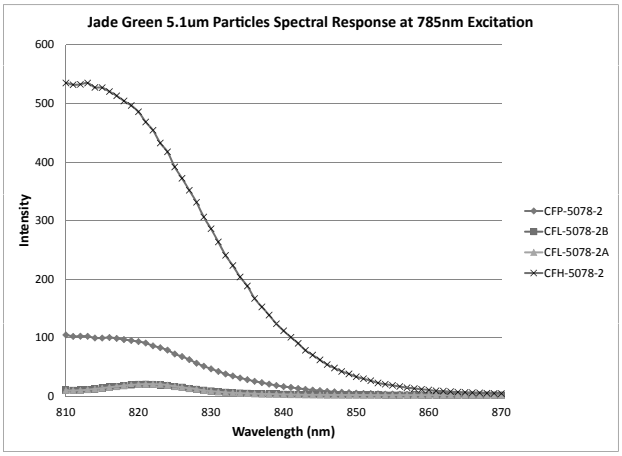
(B)



SPHERO™ Carboxyl Fluorescent IR Flow Cytometer Grade Particles

Particle Type and Surface	Size, µm	Catalog No.	Unit
Carboxyl, Fluorescent, Aqua Green, $2.9x10^{10}$/mL	0.1-0.3	CFP01-0279-10	10 mL
Carboxyl, Fluorescent, Aqua Green, $1.8x10^{9}$/mL	1.0-1.9	CFP01-1079-3	3 mL
Carboxyl, Fluorescent ,CyGreen 10^{7}/mL	3.0-3.4	CFP-3074-2	2 mL
Carboxyl, Fluorescent, Aqua Green, 10^{7}/mL	3.0-3.4	CFP-3079-2	2 mL
Carboxyl, Fluorescent, UltraBlue, 10^{7}/mL	3.5-3.9	CFP-3571-2	2 mL
Carboxyl, Fluorescent, CyGreen, 10^{7}/mL	3.5-3.9	CFP-3574-2	2 mL
Carboxyl, Fluorescent, Jade Green, 10^{7}/mL	3.5-3.9	CFP-3578-2	2 mL
Carboxyl, Fluorescent, Aqua Green, 10^{7}/mL	3.5-3.9	CFP-3579-2	2 mL
Carboxyl, Fluorescent, UltraBlue, 10^{7}/mL	5.0-5.9	CFP-5071-2	2 mL
Carboxyl, Fluorescent, CyGreen, 10^{7}/mL	5.0-5.9	CFP-5074-2	2 mL
Carboxyl, Fluorescent, Jade Green, 10^{7}/mL	5.0-5.9	CFP-5078-2	2 mL
Carboxyl, Fluorescent, Jade Green, Low Intensity Peak I, 10^{7}/mL	5.0-5.9	CFL-5078-2A	2 mL
Carboxyl, Fluorescent, Jade Green, Low Intensity Peak 2,10^{7}/mL	5.0-5.9	CFL-5078-2B	2 mL
Carboxyl, Fluorescent, Aqua Green, 10^{7}/mL	5.0-5.9	CFP-5079-2	2 mL
Carboxyl, Fluorescent, Jade Green, High Intensity, 10^{7}/mL	5.0-5.9	CFH-5078-2	2 mL

Figure 83 Spectra of Cat. No. CFP-5078-2, CFL-5078-2A, CFP-5078-2B & CFH-5078-2 at 785 nm excitation.



SPHERO™ Flow Cytometry Nano Fluorescent Size Standard Kit

- Consists of fluorescent beads with 4 different diameters from 220nm to 1.33µm
- Designed to characterize microparticles (MPs, 0.5-0.9µm), aquatic bacteria (0.2-0.6µm), and platelets (0.9-3µm)
- Used to define optimal settings for MP analysis in new cytometers with increased Forward Scatter sensitivity
- Provides a submicron size standardization tool for flow cytometers
- An addition 130nm population is available as Catalog Number NFPPS-0152-5

Particle Type and Surface	Size, µm	Catalog No.	Unit
Nano Fluorescent Size Standard Kit, Flow Cytometry Grade, Yellow, 10^6/mL	0.1-0.3 µm, 0.4-0.6 µm, 0.7-0.9 µm, &1.0-1.9 µm	NFPPS-52-4K	4x5 mL
Nano Fluorescent Size Standard, Flow Cytometry Grade, Yellow, 10^6/mL	0.05-0.15 µm,	NFPPS-0152-5	5 mL

Figure 84 SSC vs FITC Dot Plot using SSC trigger for Cat. No. NFPPS-4K, Lot AC01 on a Stratedigm S1400.

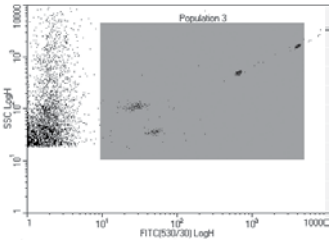


Figure 85 Dot Plots and Histograms of Cat. No. NFPPS-4K, Lot AC01, Peak 1, 0.22µm on a BD LSR2.

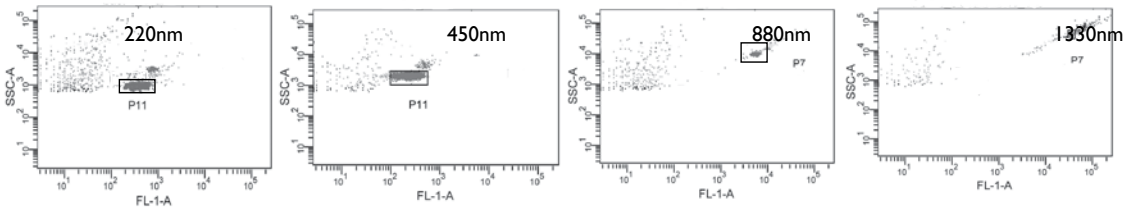


Figure 86 Cat. No. NFPPS-4K & NFPPS-0152-5 on a Beckman Coulter Cyan ADP

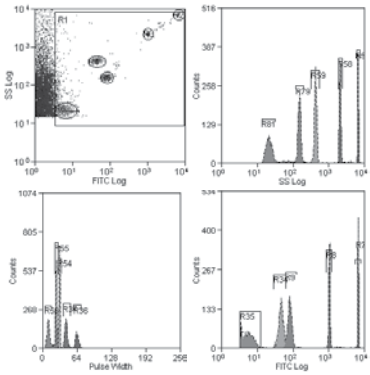


Figure 87 Dot Plots of Cat. No. NFPPS-4K, Lot AC01, on a Beckman Coulter XL.

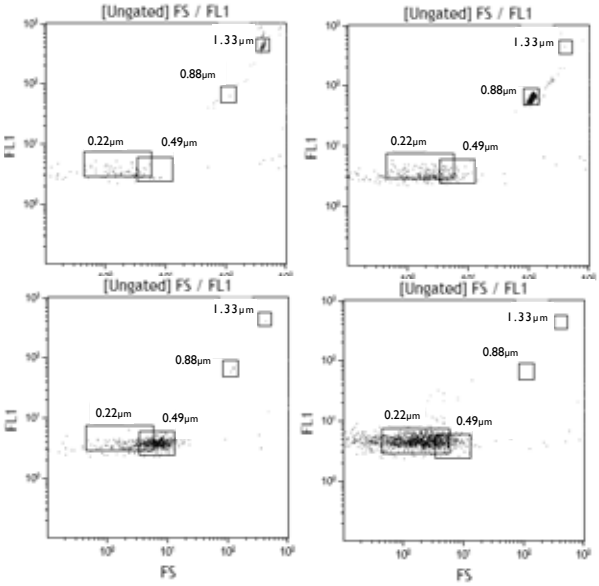
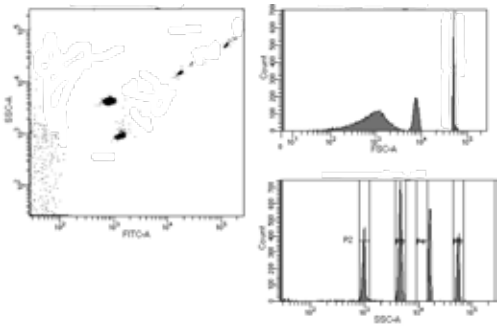


Figure 88 Cat. No. NFPPS-4K on a BD Fortessa X-20



SPHERO™ Flow Cytometry Multiplex Bead Assay Particles

SPHERO™ Flow Cytometry Multiplex Bead Assay Particles are designed for the development of flow cytometer multiplex assays. These kits are used by companies or laboratories which develop assays for allergy testing, autoimmune diseases, cardiac markers, cytokine detection, endocrine markers, infectious disease markers, isotyping, genotyping, kinase and phosphorylated protein activity, metabolic markers, and tissue typing. For example, S. Chew, et al. "Stability Screening of Arrays of Major Histocompatibility Complexes on Combinatorially Encoded Flow Cytometry Beads ." J. Biol. Chem. 2011 286: 28466-28475. These kits are available with a variety of functionality, fluorophores and coatings.

SPHERO™ Blue Fluorescent Particle Array Kits (PAK)

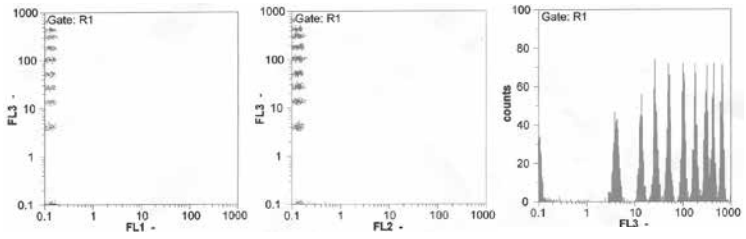
- Designed to simplify flow cytometer multiplex assay development
- Consists of fluorescent particles of different intensities in PE-Cy5 channel and minimal fluorescence in FITC and PE channels with 488 nm excitation
- Used with FITC and/or PE for detection
- Several sizes can be used independently or mixed together.
- Avaliable as Functionalized, Streptavidin, Biotin and antibody coated.

Blue PAK (**P**article **A**rray **K**its) for multiplex flow assay are now available from Spherotech. The Blue PAK particles are fluorescent in PE-Cy5, APC, and APC-Cy7 channel with minimal fluorescent in FITC and PE channels with 488 or 635 nm excitation. This allows that either FITC and/or PE tracers can be used for detection. These kits are available with different functionalities and coatings. Carboxyl, amino and plain polystyrene functionalized particles are available for covalent attachment or passive adsorption of ligands.

Particle Type and Surface	Size, µm	Catalog No.	Unit
Blue, 7 peaks, 10^8/mL	3.5-3.9	PAK-3567-7K	7x1 mL
Blue, 8 peaks, 10^8/mL	4.0-4.9	PAK-4067-8K	8x1 mL
Blue, 10 peaks, 10^8/mL	5.0-5.9	PAK-5067-10K	10x1 mL
Blue, Odd # peaks, 10^8/mL	5.0-5.9	PAK-5067-5A	5x1 mL
Blue, Even # peaks, 10^8/mL	5.0-5.9	PAK-5067-5B	5x1 mL
Blue, 9 peaks, 10^7/mL	7.0-7.9	PAK-7067-9K	9x1 mL
Carboxyl Blue, 7 peaks, 10^8/mL	3.0-3.4	CPAK-3067-7K	7x1 mL
Carboxyl Blue, 4 peaks, 10^8/mL	3.5-3.9	CPAK-3567-4K	4x1 mL
Carboxyl Blue, 7 peaks, 10^8/mL	3.5-3.9	CPAK-3567-7K	7x1 mL
Carboxyl Blue, 8 peaks, 10^8/mL	4.0-4.9	CPAK-4067-8K	8x1 mL
Carboxyl Blue, 10 peaks, 10^8/mL	5.0-5.9	CPAK-5067-10K	10x1 mL
Carboxyl Blue, Odd # peaks, 10^8/mL	5.0-5.9	CPAK-5067-5A	5x1 mL
Carboxyl Blue, Even # peaks, 10^8/mL	5.0-5.9	CPAK-5067-5B	5x1 mL
Carboxyl Blue, 9 peaks, 10^7/mL	7.0-7.9	CPAK-7067-9K	9x1 mL
Carboxyl Blue, 13 peaks, 10^7/mL	10-14.	CPAK-10067-13K	13x1 mL
Carboxyl Blue Array Chemistry Development Particles, 10^8/mL	5.0-5.9	CFP-5067-2	2 mL
Amino Blue, 7 peaks, 10^8/mL	3.5-3.9	APAK-3567-7K	7x1 mL
Goat anti-Mouse IgG Blue, 10 peaks, 10^6/mL	5.0-5.9	MPAK-5067-10K	10x1 mL
Goat anti-Mouse IgG Blue, Odd # peaks, 10^6/mL	5.0-5.9	MPAK-5067-5A	5x1 mL
Goat anti-Mouse IgG Blue, Even # peaks, 10^6/mL	5.0-5.9	MPAK-5067-5B	5x1 mL
Glutathione Blue, , 4 peaks, 10^7/mL	5.0-5.9	GSHPAK-5067-4K	4x1 mL
Streptavidin Blue, 10 peaks, 10^6/mL	5.0-5.9	SVPAK-5067-10K	10x1 mL
Streptavidin Blue, Odd # peaks, 10^6/mL	5.0-5.9	SVPAK-5067-5A	5x1 mL

Most state-of-the-art single laser flow cytometers can resolve the 3.6 µm, 4.0 µm and 5.1µm Blue PAKs easily in FSC/SSC channels. Ideally, one can mix them together and use both FITC and PE to run 50 assays in the same tubes with most single laser flow cytometers.

Figure 89 Histograms of Cat. No. PAK-5067-10K (Blue PAK, 5.1 µm, 10 peaks) provided by Partec GmbH.



SPHERO™ Fluorescent Particle Array Kits (PAK)

The Yellow Fluorescent Particle Kits consist of 2.8 µm and 3.6 µm particles with multiple fluorescence intensities in FITC channels. These kits are used to develop multiplex assays using either PE or PE-Cy5 tracers for detection.

The Pink Fluorescent Particle Kits consist of either 2.8 µm or 3.6 µm with twelve different intensities in the PE channel. FITC conjugates can be used for detection. They are supplied as kits of either six odd numbered peaks or six even numbered peaks for the 2.8 µm and 3.6 µm particle kits. Carboxyl Pink Fluorescent are available for covalent coupling of proteins, antibodies, or antigens. Goat anti-Mouse IgG (Fc), Streptavidin, and Biotin coated Pink Fluorescent Particles Kits are also available for other applications.

The Pink Fluorescent Particle Array Kits (FPAK) are similar to the Pink Fluorescent Particle Kits except that the FPAK particles are made from a higher quality polystyrene particle. These particles are designed for flow cytometry application. The 3.2 µm kit has nine different intensities, while the 4.3 µm kit has ten different intensities. The FPAK particles can be used in conjunction with the Blue PAKs for advanced multiplex applications.

Particle Type and Surface	Size, µm	Catalog No.	Unit
Yellow, Odd # peaks, 0.5% w/v	2.5-2.9	FA-2552-6K	6x1 mL
Yellow, Even # peaks, 0.5% w/v	2.5-2.9	FB-2552-6K	6x1 mL
Pink, 9 peaks, 10^8/mL	3.0-3.4	FPAK-3058-9K	9x1 mL
Pink, Odd # peaks, 0.5% w/v	3.5-3.9	FA-3558-6K	6x1 mL
Pink, Even # peaks, 0.5% w/v	3.5-3.9	FB-3558-6K	6x1 mL
Pink, 10 peaks, 10^8/mL	4.0-4.5	FPAK-4058-10K	10x1 mL
Yellow, 9 peaks, 0.5% w/v	3.5-3.9	FX-3552-9K	9x1 mL
Carboxyl Yellow, Odd # peaks, 0.25% w/v	2.5-2.9	CFA-2552-6K	6x1 mL
Carboxyl Yellow, Even # peaks, 0.25% w/v	2.5-2.9	CFB-2552-6K	6x1 mL
Carboxyl Yellow, 9 peaks, 0.25% w/v	3.5-3.9	CFX-3552-9K	9x1 mL
Carboxyl Yellow, 3 peaks, 10^7/mL	7.0-7.9	CPAK-7052-3K	3x1 mL
Carboxyl Yellow, 6 peaks, 10^7/mL	7.0-7.9	CPAK-7052-6K	6x1 mL
Carboxyl Pink, Odd # peaks, 0.25% w/v	2.5-2.9	CFA-2558-6K	6x1 mL
Carboxyl Pink, Even # peaks, 0.25% w/v	2.5-2.9	CFB-2558-6K	6x1 mL
Carboxyl Pink, Odd # peaks, 0.25% w/v	3.5-3.9	CFA-3558-6K	6x1 mL
Carboxyl Pink, Even # peaks, 0.25% w/v	3.5-3.9	CFB-3558-6K	6x1 mL
Goat anti-Mouse IgG (Fc), Pink, Odd # peaks, 0.1% w/v	3.5-3.9	MFA-3558-6K	6x1 mL
Goat anti-Mouse IgG (Fc), Pink, Even # peaks, 0.1% w/v	3.5-3.9	MFB-3558-6K	6x1 mL
Streptavidin Yellow, Odd # peaks, 0.1% w/v	2.5-2.9	SVFA-2552-6K	6x1 mL
Streptavidin Yellow, Even # peaks, 0.1% w/v	2.5-2.9	SVFB-2552-6K	6x1 mL
Streptavidin Pink, Odd # peaks, 0.1% w/v	2.5-2.9	SVFA-2558-6K	6x1 mL
Streptavidin Pink, Even # peaks, 0.1% w/v	2.5-2.9	SVFB-2558-6K	6x1 mL
Biotin Pink, Odd # peaks, 0.1% w/v	3.5-3.9	TFA-3558-6K	6x1 mL
Biotin Pink, Even # peaks, 0.1% w/v	3.5-3.9	TFB-3558-6K	6x1 mL

Figure 90 SSC vs FSC histograms of Cat. Nos. FPAK-4058-10K, FPAK-3058-9K, FA-3558-6K and FB-3558-6K.

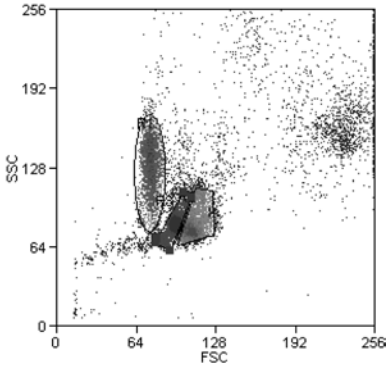
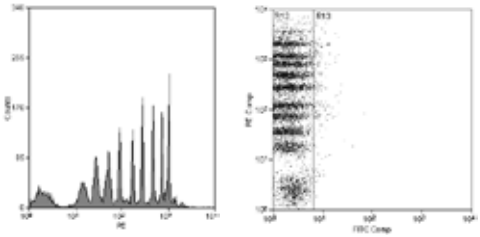


Figure 91 Histograms of Cat. No. FPAK-4058-10K (Fluorescent Particle Array Kit, Pink, 10 peaks, 4.3 µm, 1E8/mL, 10x1 mL).



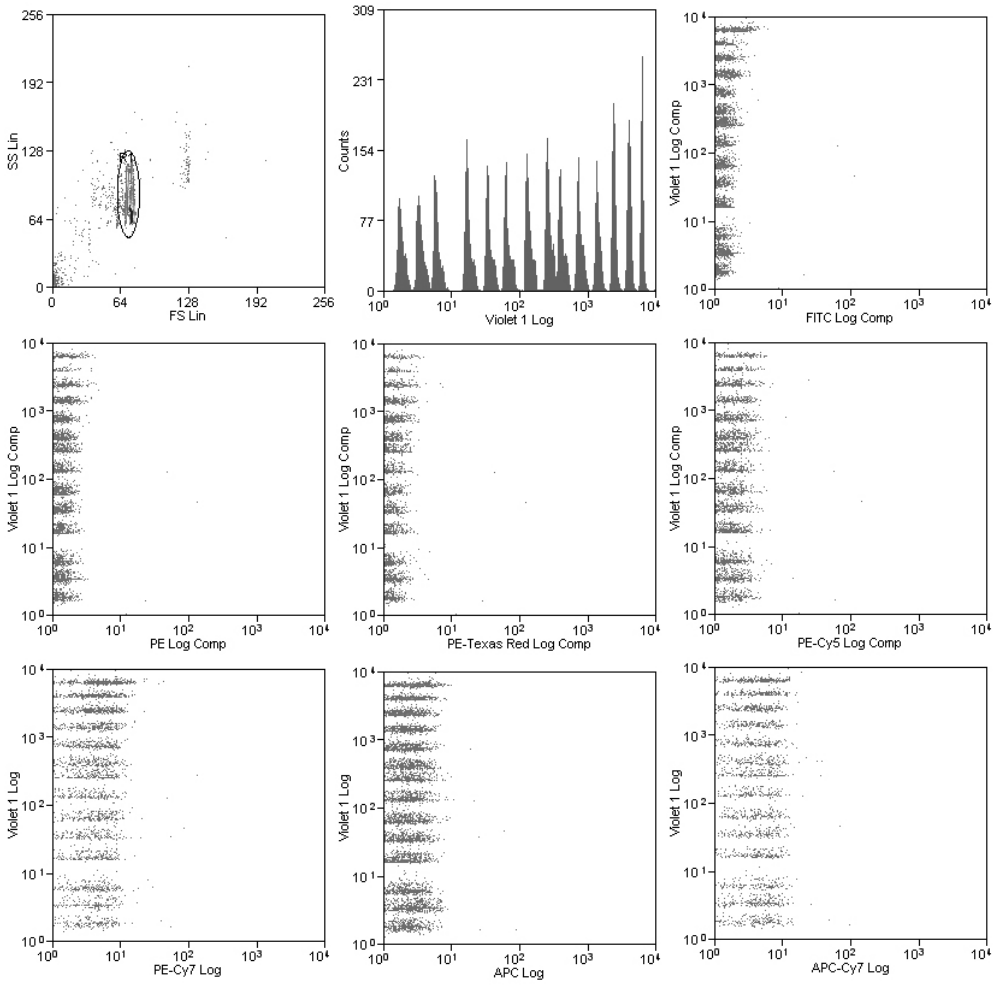
SPHERO™ UV Carboxyl Particle Array Kits (UVCPAK)

- Designed for multiplex flow assays on commercially available flow cytometers with UV or Violet excitation
- Detected in the UV and Violet channels
- Display minimal fluorescence in channels with 488 nm, 532 nm, and 633 nm excitation
- Available in 5.0 µm, 14 peaks at $1x10^8$ particles/mL
- Carboxylated for the attachment of your specific antibodies.

Particle Type and Surface	Size, µm	Catalog No.	Unit
Carboxyl UV, 14 peaks, 10^8/mL	5.0-5.9	UVCPAK-5042-14K	14x1 mL

The UVCPAK allows the expansion of multiplex assays with additional detection channels. Analytes of interest can be simultaneously quantified using flow cytometric analysis and any fluorescent conjugates with 488 nm, 532 nm, or 633 nm excitation.

Figure 92 Histograms of Cat. No. UVCPAK-5042-14K (UV Carboxyl Particle Array Kit, 14 peaks, 1E8/mL, 5.0-5.9 um, 14x1 mL).



SPHERO™ Fluorescent Particle Slides

- Aids in the routine alignment and calibration of Laser Scanning Cytometers, confocal fluorescent microscopes and other fluorescent imaging systems
- Used to determine the sensitivity and system performance
- Many contain a mixture of fluorophores and intensities which allows detection in multiple channels.

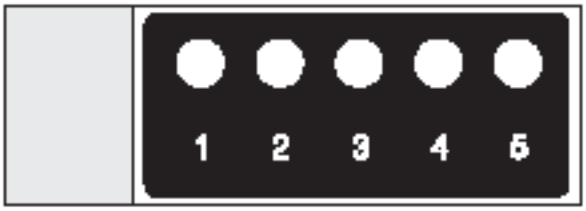
The Fluorescent Particle Slides are designed for routine alignment and calibration of Laser Scanning Cytometers, confocal fluorescent microscopes and other fluorescent imaging systems. The slides are prepared by mounting Fluorescent Particles or Rainbow Fluorescent Particles in a permanent mounting medium.

We can mount other fluorescent or non-fluorescent particles on slides. Please contact us for custom mounting of

Particle Type and Surface	Size, µm	Catalog No.	Unit
Rainbow Fluorescent Particle Slide, 5 Intensities, For linear scale	3.0-3.4	FPS-3057-5	each
Rainbow Fluorescent Particle Slide, 6 Intensities, For log scale	3.0-3.4	FPS-3057-6	each
Rainbow Fluorescent Particle Slide, 5 Intensities in individual wells, For linear scale	3.0-3.4	FPS-3057-5LN	each
Rainbow Fluorescent Particle Slide, 5 Intensities in individual wells, For log scale	3.0-3.4	FPS-3057-5LG	each
Rainbow Fluorescent Particle Slide	5.0-5.9	FPS-5057	each
Rainbow Fluorescent Particle Slide, 3 Intensities	6.0-6.4	FPS-6057-3	each
Rainbow Fluorescent Particle Slide	10.0-14.0	FPS-10057	each
Rainbow Fluorescent Particle Slide	13.0-17.9	FPS-15057	each
Rainbow Fluorescent Particle Slide, 4 Intensities	13.0-17.9	FPS-15057-4	each
Rainbow Fluorescent Particle Slide, Level 5 Intensity	13.0-17.9	FPS-15057-L5	each
Rainbow Fluorescent Particle Slide, 6 Sizes	0.5-15.0	FPS-M57-6	each
Ultra Rainbow Fluorescent Particle Slide	5.0-5.9	FPS-5057-UR	each
Ultra Rainbow Fluorescent Particle Slide, 5 Intensities	5.0-5.9	FPS-5057-UR5	each
Fluorescent UV Particle Slide	5.0-5.9	FPS-5040	each
Fluorescent Yellow Particle Slide	5.0.5.9	FPS-5052	each
Fluorescent Nile Red Particle Slide	5.0-5.9	FPS-5056	each
Fluorescent Sky Blue Particle Slide	5.0.-5.9	FPS-5070	each
Fluorescent UV Particle Slide	10.0-14.0	FPS-10040	each
Fluorescent Yellow Particle Slide	10.0-14.0	FPS-10052	each
Fluorescent Nile Red Particle Slide	10.0-14.0	FPS-10056	each
Fluorescent Purple Particle Slide	10.0-14.0	FPS-10062	each
Fluorescent Sky Blue Particle Slide	10.0-14.0	FPS-10070	each
Fluorescent Sky Blue Particle Slide, 4 different fluorescent; UV, Yellow, Nile Red, and Blue	10.0-14.0	FPS-100M4	each
Fluorescent Sky Blue Particle Slide, 4 different fluorescent; UV, Yellow, Nile Red, and Purple	10.0-14.0	FPS-100M4B	each
Mirror Slide	n/a	MRS-1	each

Figure 93 Standard 25 mm x 75 mm x 1 mm slide used during the manufacturing of 17 x 24 mm oblong, single well fluorescent particle slides.

Figure 94 Standard 25 mm x 75 mm x 1 mm slide used during the manufacturing of 6 mm, 5 well fluorescent particle slides.



SPHERO™ Magnetic Particles

- SPHERO™ Magnetic Microparticles provide high quality and reproducible results for your application
- Allow for rapid and reliable binding between the target and magnetic particle
- Consists of a uniform, monodispersed surface for optimal performance.

The SPHERO™ Magnetic Particles (Paramagnetic Particles) are prepared by coating a layer of iron oxide and polystyrene onto polystyrene core particles. The SPHERO™ Magnetic Particles are relatively uniform in size, spherical in shape and paramagnetic in nature. The paramagnetic nature of the particles allows them to be separated using a magnet and resuspended easily when removed from the magnet. They do not retain any significant magnetism even after repeat exposure to strong magnetic fields.

The SPHERO™ Smooth Surface Magnetic Particles have a thick layer of polymer coating on the surface of the particles to fully encapsulate the iron oxide coating. There is no exposed iron oxide on the surface of the particles. These particles are paramagnetic. The SPHERO™ Smooth Surface Magnetic Particles are particularly useful in applications where exposed iron oxide may interfere with the enzymatic activities or cause other undesirable interferences. The SPHERO™ Magnetic Particles are used for cell separation, affinity purification, DNA probe assays, magnetic particle EIA, etc.

The SPHERO™ Cross-linked Magnetic Particles are prepared to render them resistant to common organic solvents such as acetone, acetonitrile, DMF and chloroform. The cross-linked magnetic particles have significantly greater surface area and higher magnetite content (~15%) compared to the 4.0 µm uniform magnetic particles. They are also paramagnetic. Large surface area combined with higher magnetite content make SPHERO™ Cross-linked Magnetic Particles ideal solid phase for use in cell separation, magnetic removal of microorganisms, viruses and cross reactants in serum, as well as, affinity purification applications.

Figure 95 Histograms of Cat. No. CM-60-10 (Carboxyl Magnetic Particles, 2.5% w/v, 7.1 um, 10 mL).

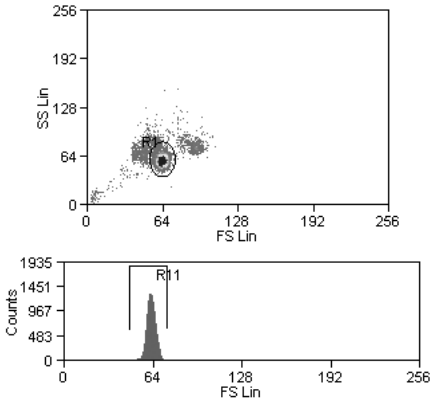


Figure 96 SEM Photo of Spherotech Cat. No. CM-80-10 (Carboxyl Magnetic Particles, 1% w/v, 8.5 um, 10 mL).

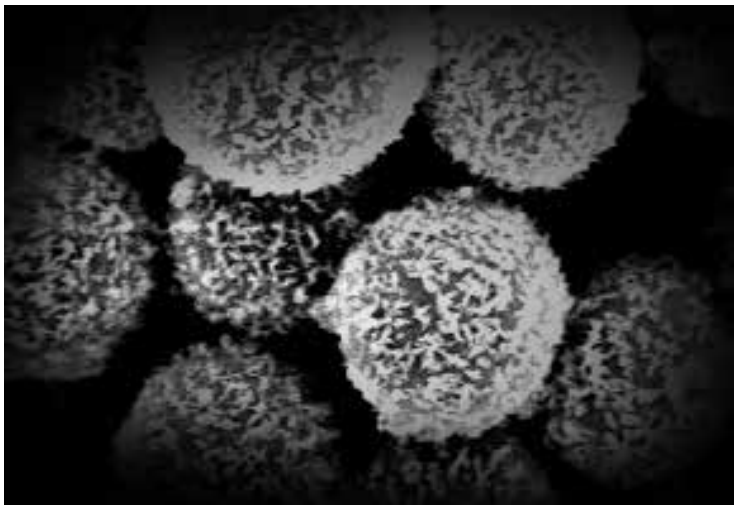
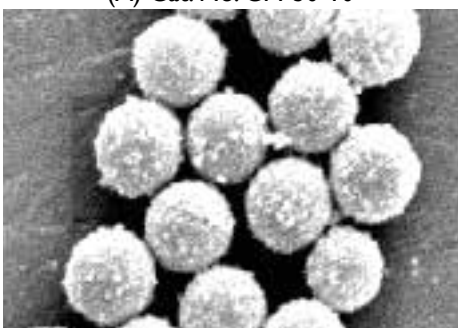
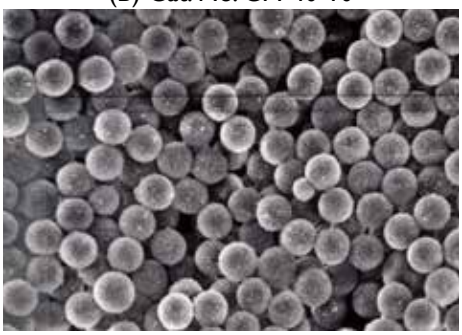


Figure 97 Representative Scanning Electron Microscope photos of SPHERO™ Magnetic Particles are shown below: (A) CM-30-10 at 2000X, (B) CM-40-10 at 1000X, (C) Cross section of SPHERO™ Magnetic Particles, dark specks in (C) are magnetite on the surface of core particles. The SEM photo of SPHERO™ Smooth Surface Magnetic Particles, CMS-40-10, is shown in (D) at 5000x.

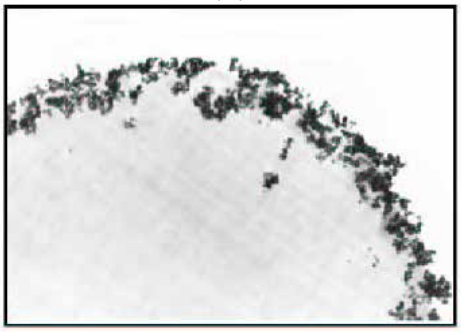
(A) Cat. No. CM-30-10



(B) Cat. No. CM-40-10



(C)



(D) Cat. No. CMS-40-10

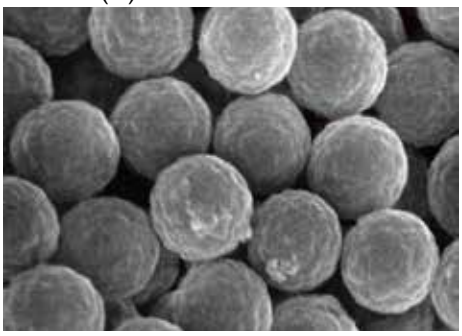
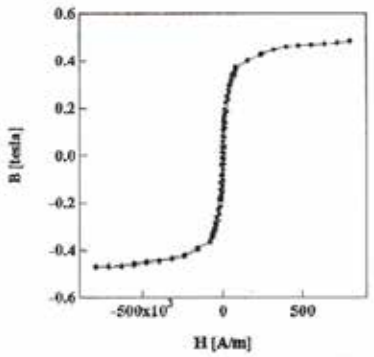


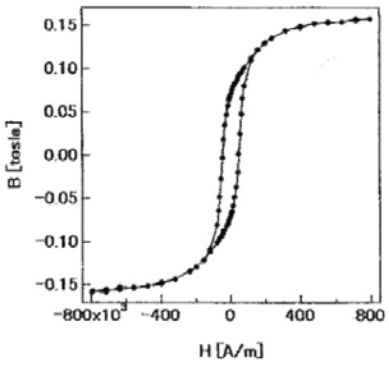
Figure 98 Magnetic Characteristics of SPHERO™ Magnetic Particles and Ferromagnetic Particles.

The magnetic characteristics of magnetic particles are determined by measuring the magnetic Hysteresis Loop of magnetic particles with a magnetometer as shown below. The magnetic particles are subjected to an increasing magnetizing field (H in Oersteds) in one direction, while sensing the magnetic field (B in Gauss) in the sample to reach maximum or saturation magnetization (Bm). The magnetizing field is then returned to zero and the field retained is measured as the remnant magnetization (Br). Finally, the field is reversed until magnetization is at zero again. The corresponding field strength (Hc) is the coercivity of the magnetic particles. If the Br and Hc are near zero, the magnetic particles are characterized as superparamagnetic as shown in (A) . On the other hand, the Ferromagnetic Particles will have Hysteresis Loop similar to (B).

(A) Cat. No. CM-10-10 showing superparamagnetic characteristics.



(B) Cat. No. CFM-40-10 showing ferromagnetic characteristics.



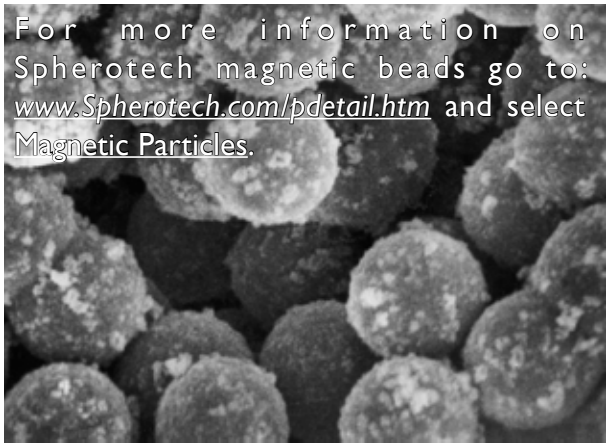
SPHERO™ Magnetic Polystyrene Particles

- Consits of paramagnetic particles prepared by coating a layer of iron oxide and polystyrene onto polystyrene core particles
- Uniform in size and spherical in shape
- Separated using a magnet and resuspended when removed from the magnetic field
- Used for cell separation, affinity purification, DNA probe assays, magnetic particle EIA, etc.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Magnetic Polystyrene	2.0-2.9	2.5	PM-20-10	10 mL
Magnetic Polystyrene	2.0-2.9	2.5	PM-20-100	100 mL
Magnetic Polystyrene	3.0-3.9	2.5	PM-30-10	10 mL
Magnetic Polystyrene	3.0-3.9	2.5	PM-30-100	100 mL
Magnetic Polystyrene	4.0-4.5	2.5	PM-40-10	10 mL
Magnetic Polystyrene	4.0-4.5	2.5	PM-40-100	100 mL
Magnetic Polystyrene	5.0-5.9	2.5	PM-50-10	10 mL
Magnetic Polystyrene	5.0-5.9	2.5	PM-50-100	100 mL

SPHERO™ Carboxyl Magnetic Particles

- Used during the isolation and affinity purification of biomolecules in a wide range of assays and applications
- Contain carboxylic acid groups which can be used for carbodiimide activation (e.g. EDC) for covalent coupling
- Couple to the primary amino groups of nucleic acids, peptides, proteins or other target molecules.



Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Carboxyl Magnetic	0.1-0.39	2.5	CM-025-10	10 mL
Carboxyl Magnetic	0.4-0.69	2.5	CM-05-10	10 mL
Carboxyl Magnetic	0.7-0.9	2.5	CM-08-10	10 mL
Carboxyl Magnetic	1.0-1.4	2.5	CM-10-10	10 mL
Carboxyl Magnetic	1.0-1.4	2.5	CM-10-100	100 mL
Carboxyl Magnetic	1.5-1.9	2.5	CM-15-10	10 mL
Carboxyl Magnetic	1.5-1.9	2.5	CM-15-100	100 mL
Carboxyl Magnetic	2.0-2.9	2.5	CM-20-10	10 mL
Carboxyl Magnetic	2.0-2.9	2.5	CM-20-100	100 mL
Carboxyl Magnetic	3.0-3.9	2.5	CM-30-10	10 mL
Carboxyl Magnetic	3.0-3.9	2.5	CM-30-100	100 mL
Carboxyl Magnetic	4.0-4.5	2.5	CM-40-10	10 mL
Carboxyl Magnetic	4.0-4.5	2.5	CM-40-100	100 mL
Carboxyl Magnetic	5.0-5.9	2.5	CM-50-10	10 mL
Carboxyl Magnetic	6.0-8.0	2.5	CM-60-10	10 mL
Carboxyl Magnetic	6.0-8.0	2.5	CM-60-100	100 mL
Carboxyl Magnetic	8.0-9.9	2.5	CM-80-10	10 mL
Carboxyl Magnetic	10.0-13.9	1.0	CM-100-10	10 mL
Carboxyl Magnetic	14.0-17.9	1.0	CM-150-10	10 mL
Carboxyl Magnetic	18.0-22.9	1.0	CM-200-10	10 mL
Carboxyl Magnetic	27.0-37.0	1.0	CM-300-10	10 mL
Carboxyl Magnetic	90.0-120.0	1.0	CM-1000-10	10 mL

SPHERO™ Jeffamine® Magnetic Particles

Contains a PEG-based spacer arm that is terminated with amine groups for coupling carboxyl-containing molecules.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Jeffamine® Magnetic	0.1-0.39	1.0	JAM-025-10	10 mL
Jeffamine® Magnetic	3.0-3.9	2.5	JAM-30-10	10 mL
Jeffamine® Magnetic, Smooth Surface	3.0-3.9	2.5	JAMS-30-10	10 mL

JEFFAMINE® is a registered trademark of Huntsman Corporation

SPHERO™ Amino Magnetic Particles

- Supplied as an aqueous suspension of magnetic iron oxide particles coated to provide primary amino groups
- Used to covalently couple proteins using bifunctional crosslinking agents
- Rapidly separates bound from unbound molecules using a magnetic separator due to paramagnetic properties.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Amino Magnetic	1.0-1.4	2.5	AM-10-10	10 mL
Amino Magnetic	1.0-1.4	2.5	AM-10-100	100 mL
Amino Magnetic	1.5-1.9	2.5	AM-15-10	10 mL
Amino Magnetic	1.5-1.9	2.5	AM-15-100	100 mL
Amino Magnetic	2.0-2.9	2.5	AM-20-10	10 mL
Amino Magnetic	2.0-2.9	2.5	AM-20-100	100 mL
Amino Magnetic	3.0-3.9	2.5	AM-30-10	10 mL
Amino Magnetic	3.0-3.9	2.5	AM-30-100	100 mL
Amino Magnetic	4.0-4.9	2.5	AM-40-10	10 mL
Amino Magnetic	4.0-4.9	2.5	AM-40-100	100 mL
Amino Magnetic	6.0-6.9	1.0	AM-60-10	10 mL
Amino Magnetic	6.0-6.9	1.0	AM-60-100	100 mL
Amino Magnetic	8.0-9.9	1.0	AM-80-10	10 mL

SPHERO™ Magnetic Cross-linked Particles

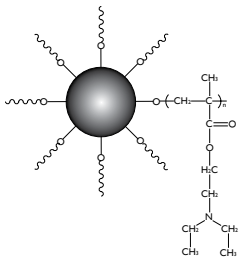
- Contains a very high magnetic content for fast magnetic response time
- Have high binding capacities due to their large surface area
- Resistant to common organic solvents such as acetone, acetonitrile, DMF and chloroform
- Used in a wide variety of molecular biology, nucleic acid isolation, research protocols and clinical immunoassay reagent applications.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Magnetic, Cross-linked, granules, non-uniform	3.0-6.0	2.5	PMX-40-10	10 mL
Amino Magnetic, Cross-linked	1.0-2.0	2.5	AMX-10-10	10 mL
Amino Magnetic, Cross-linked	1.0-2.0	2.5	AMX-10-100	100 mL
Amino Magnetic, Cross-linked	3.0-3.9	2.5	AMX-30-10	10 mL
Amino Magnetic, Cross-linked, granules, non-uniform	3.0-6.0	2.5	AMX-40-10	10 mL
Amino Magnetic, Cross-linked	13.0-17.99	1.0	AMX-150-5	5 mL
Carboxyl Magnetic, Cross-linked	1.0-2.0	2.5	CMX-10-10	10 mL
Carboxyl Magnetic, Cross-linked	1.0-2.0	2.5	CMX-10-100	100 mL
Carboxyl Magnetic, Cross-linked, granules, non-uniform	3.0-6.0	2.5	CMX-40-10	10 mL

SPHERO™ Diethylamino Magnetic Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Diethylamino Magnetic	3.0-3.9	2.5	DEM-30-10	10 mL

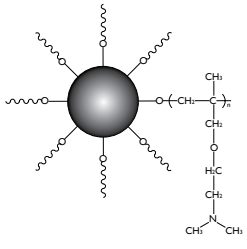
Figure 99 Illustration of a DEAEMA magnetic particle.



SPHERO™ Dimethylamino Magnetic Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Dimethylamino Magnetic	3.0-3.9	2.5	DM-30-10	10 mL

Figure 100 Illustration of a DMAEMA magnetic particle.



SPHERO™ Magnetic Polystyrene Particles, Smooth Surface

- Consists of a thick layer of polymer coating on the surface to encapsulate the iron oxide coating
- No exposed iron oxide on the surface
- Used in applications where exposed iron oxide causes undesirable interferences.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Magnetic Polystyrene , Smooth Surface	2.0-2.9	2.5	PMS-20-10	10 mL
Magnetic Polystyrene , Smooth Surface	2.0-2.9	2.5	PMS-20-100	100 mL
Magnetic Polystyrene , Smooth Surface	3.0-3.9	2.5	PMS-30-10	10 mL
Magnetic Polystyrene , Smooth Surface	3.0-3.9	2.5	PMS-30-100	100 mL
Magnetic Polystyrene , Smooth Surface	4.0-5.0	2.5	PMS-40-10	10 mL
Magnetic Polystyrene , Smooth Surface	4.0-5.0	2.5	PMS-40-100	100 mL

SPHERO™ Amino Magnetic Particles, Smooth Surface

- NEW - SPHERO™ Amino High Magnetic Content Microparticles
- Aids in the separation in whole blood
- Monodispersed surface for optimal performance.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Amino Magnetic, Smooth Surface	4.0-5.0	2.5	AMS-40-10	10 mL
Amino Magnetic, Smooth Surface	4.0-5.0	2.5	AMS-40-100	100 mL
Amino Magnetic, Smooth Surface, High Iron	4.0-5.0	2.5	AMS-40-10H	10 mL

SPHERO™ Carboxyl Magnetic Particles, Smooth Surface

- Activated for covalent coupling to amine-containing molecules using a variety of mechanisms
- Used with one or two-step coupling using EDC to form amide bonds with proteins or other molecules
- Used for coupling to amino modified oligonucleotide probes with MES buffer and EDC.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Carboxyl Magnetic, Smooth Surface	3.0-3.9	2.5	CMS-30-10	10 mL
Carboxyl Magnetic, Smooth Surface	3.0-3.9	2.5	CMS-30-100	100 mL
Carboxyl Magnetic, Smooth Surface	4.0-5.0	2.5	CMS-40-10	10 mL
Carboxyl Magnetic, Smooth Surface	4.0-5.0	2.5	CMS-40-100	100 mL
Carboxyl Magnetic, Smooth Surface	8.0-9.9	1.0	CMS-80-10	10 mL
Carboxyl Magnetic, Smooth Surface	18.0-22.9	1.0	CMS-200-10	10 mL

SPHERO™ Hydroxyethyl Magnetic Particles, Smooth Surface

- Contains primary hydroxyls on the surface
- Exhibits hydrophilic characteristics
- Activated for covalent coupling using epoxy and vinyl sulfone activation procedures
- Used for the coupling to amine-, thiol-, or hydroxyl-containing ligands.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Hydroxyethyl Magnetic, Smooth Surface	3.0-3.9	2.5	HEMS-30-10	10 mL

SPHERO™ Ferromagnetic Particles

- Manufactured by coating a layer of chromium dioxide and polystyrene onto polystyrene core particles
- Retains magnetism once exposed to a magnetic field
- Exhibits a higher magnetic moment then paramagnetic particles
- Have been used for magnetic twisting cytometry, microfluidics, and cellular labeling.

Unlike paramagnetic particles that are made using iron oxide, SPHERO™ Ferromagnetic Particles are prepared using chromium dioxide coated onto uniform polystyrene particles. These particles retain magnetism once exposed to a magnetic field. The particles can be demagnetized and re-magnetized repeatedly and reproducibly. Ferromagnetic particles have been used for studying mechanotransduction across the cell surface and through the cytoskelaton. This is performed by binding them to cell surface receptors and applying mechanical stress directly to the receptor using a device to twist the magnetic particle.

SPHERO™ Amino Ferromagnetic Particles

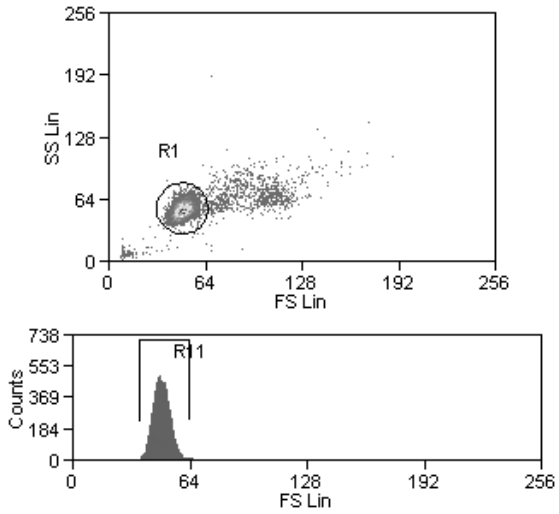
Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Amino Ferromagnetic	4.0-4.5	1.0	AFM-40-10	10 mL

Hystersis data of Cat. No. AFM-40-10 ferromagnetic beads measured at 285, 250 and 200K under a maximum applied field of 4kOe has been reported by De Los Santos V, L., J. Llandro, et al. (2009), "Magnetic measurements of suspended functionalised ferromagnetic beads under DC applied fields." *Journal of Magnetism & Magnetic Materials* 321(14): 2129-2134.

SPHERO™ Carboxyl Ferromagnetic Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Carboxyl Ferromagnetic	2.0-2.9	1.0	CFM-20-10	10 mL
Carboxyl Ferromagnetic	4.0-4.9	1.0	CFM-40-10	10 mL
Carboxyl Ferromagnetic	6.0-7.9	1.0	CFM-60-5	5 mL
Carboxyl Ferromagnetic	8.0-8.9	1.0	CFM-80-5	5 mL
Carboxyl Ferromagnetic	28.0-34.9	0.5	CFM-300-5	5 mL
Carboxyl Ferromagnetic	90.0-120.0	1.0	CFM-1000-5	5 mL
Carboxyl Ferromagnetic Particles, Cross-linked, granules, non-uniform	~1-2 µm	1.0	CFMX-10-10	10 mL

Figure 101 Histograms of Cat. No. CFM-40-10 (Carboxyl Ferromagnetic Particles, 1.0% w/v, 4.93 um, 10 mL).



SPHERO™ Fluorescent Carboxyl Ferromagnetic Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Fluorescent Yellow Carboxyl Ferromagnetic	2.0-2.9	1.0	FCFM-2052-2	2 mL
Fluorescent Yellow Carboxyl Ferromagnetic	4.0-4.9	1.0	FCFM-4052-2	2 mL
Fluorescent Nile Red Carboxyl Ferromagnetic	4.0-4.9	1.0	FCFM-4056-2	2 mL
Fluorescent Nile Red Carboxyl Ferromagnetic	5.0-5.9	1.0	FCFM-5056-2	2 mL
Fluorescent Yellow Carboxyl Ferromagnetic	38.0-44.0	1.0	FCFM-40052-2	2 mL

SPHERO™ Fluorescent Magnetic Particles

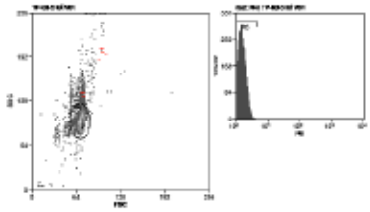
Consists of paramagnetic particles made by either staining the polystyrene core or polymerizing a fluorophore in styrene in the presence of polystyrene core particles.

SPHERO™ Fluorescent Magnetic Particles

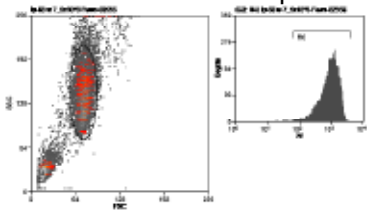
Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Fluorescent Magnetic, Nile Red,	4.0-4.9	1.0	FPM-4056-2	2 mL
Fluorescent Magnetic, UV	5.0-5.9	0.1	FPM-5041-2	2 mL

Figure 102 Histograms of the magnetic separation of Biotin beads (Cat. No. TP-60-5) after exposure to streptavidin coated carboxyl fluorescent magnetic particles, nile red (Cat. No. FSVM-02556-5).

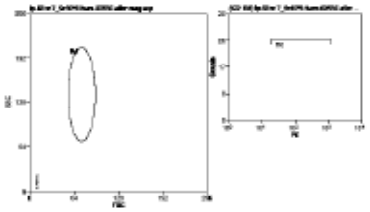
A. Biotin beads before exposure.



B. Biotin beads after exposure.



C. Biotin beads after magnetic separation.



SPHERO™ Amino Fluorescent Magnetic Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Fluorescent Amino Magnetic ,Yellow	2.0-2.9	1.0	FAM-2052-2	2 mL
Fluorescent Amino Magnetic, Pink	2.0-2.9	1.0	FAM-2058-2	2 mL
Fluorescent Amino Magnetic, Nile Red,	4.0-4.9	1.0	FAM-4056-2	2 mL
Fluorescent Jeffamine®, Magnetic, Nile Red	0.2-0.39	0.1	FJAM-02556-2	2 mL

JEFFAMINE® is a registered trademark of Huntsman Corporation

SPHERO™ Carboxyl Fluorescent Magnetic Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Fluorescent Carboxyl Magnetic, Nile Red	0.2-0.39	1.0	FCM-02556-2	2 mL
Fluorescent Carboxyl Magnetic, High Iron, Yellow	0.4-0.69	0.5	FCM-0552-2H	2 mL
Fluorescent Carboxyl Magnetic,, Nile Red	0.4-0.69	0.5	FCM-0556-2	2 mL
Fluorescent Carboxyl, Magnetic, Yellow	0.7-0.9	1.0	FCM-0852-2	2 mL
Fluorescent Carboxyl, Magnetic, Nile Red	0.7-0.9	1.0	FCM-0856-2	2 mL
Fluorescent Carboxyl Magnetic, Light Yellow	1.0-1.4	1.0	FCM-1045-2	2 mL
Fluorescent Carboxyl Magnetic, Yellow	1.0-1.4	1.0	FCM-1052-2	2 mL
Fluorescent Carboxyl Magnetic, Nile Red	1.0-1.4	1.0	FCM-1056-2	2 mL
Fluorescent Carboxyl Magnetic, Pink	1.0-1.4	1.0	FCM-1058-2	2 mL
Fluorescent Carboxyl Magnetic, Blue	1.0-1.4	1.0	FCM-1068-2	2 mL
Fluorescent Carboxyl Magnetic, Sky Blue	1.0-1.4	1.0	FCM-1070-2	2 mL
Fluorescent Carboxyl Magnetic, UV/ Light Yellow	2.0-2.4	1.0	FCM-2042-2	2 mL
Fluorescent Carboxyl Magnetic, Yellow	2.0-2.4	1.0	FCM-2052-2	2 mL
Fluorescent Carboxyl Magnetic, Pink	2.0-2.4	1.0	FCM-2058-2	2 mL
Fluorescent Carboxyl Magnetic, Purple	2.0-2.4	1.0	FCM-2062-2	2 mL
Fluorescent Carboxyl Magnetic, Sky Blue	2.0-2.4	1.0	FCM-2070-2	2 mL
Fluorescent Carboxyl Magnetic, Yellow	4.0-4.9	1.0	FCM-4052-2	2 mL
Fluorescent Carboxyl Magnetic, Nile Red	4.0-4.9	1.0	FCM-4056-2	2 mL
Fluorescent Carboxyl Magnetic, Pink	4.0-5.0	1.0	FCM-4058-2	2 mL
Fluorescent Carboxyl Magnetic, Yellow	5.0-5.9	1.0	FCM-5052-2	2 mL
Fluorescent Carboxyl Magnetic, UV	7.0-7.9	0.1	FCM-7041-2	2 mL
Fluorescent Carboxyl Magnetic, PAK Blue	7.0-7.9	0.1	FCM-7067-2	2 mL
Fluorescent Carboxyl Magnetic, Yellow	8.0-9.9	1.0	FCM-8052-2	2 mL
Fluorescent Carboxyl Magnetic, Nile Red	8.0-9.9	1.0	FCM-8056-2	2 mL
Fluorescent Carboxyl Magnetic, Pink	8.0-9.9	1.0	FCM-8058-2	2 mL
Fluorescent Carboxyl Magnetic, Yellow	10.0-14.0	1.0	FCM-10052-2	2 mL
Fluorescent Carboxyl Magnetic, Yellow	18.0-24.9	1.0	FCM-20052-2	2 mL
Fluorescent Carboxyl Magnetic, Yellow	44.0-52.9	1.0	FCM-50052-2	2 mL
Fluorescent Carboxyl Magnetic, Yellow	90-105	1.0	FCM-100052-2	2 mL
Fluorescent Carboxyl Magnetic, Yellow	180-210	1.0	FCM-200052-2	2 mL

SPHERO™ Magnetic Separators

Spherotech has several different designs of magnetic separators. These are used for separating both Paramagnetic and Ferromagnetic particles. They accommodate different size tubes and other receptacles specific to various applications.

A combination of magnetic particles and conventional enzyme immuno assay (EIA) microplate technology offers significant advantages over conventional EIA. Some of the advantages are listed below:

- Magnetic particles offer larger surface area and significantly faster reaction kinetics. As a result, the total time to complete an assay is reduced
- Microparticles are washed more efficiently leaving less residual reactants; thus, lowering background signal and potentially improving sensitivity
- Coating of magnetic particle is easier, provides a more uniform solid phase and helps to minimize lot-to-lot variation.

(A) The SPHERO™ FlexiMag Separator (Cat. No. FMS-1000) is ideal for any laboratory working with magnetic particles for cell separation, immunoassay or affinity purification. It offers the flexibility to meet the small scale requirements of a research laboratory and the large quantity processing of a commercial facility. It uses interchangeable tube holders to secure different size tubes and bottles. The separator comes with a set of three tube holders, Small, Medium and Large. Additional holders can be purchased separately,.

a) The Small holder accommodates four 1.5 mL microfuge or four 10 or 12x75 mm test tubes (8 total)
b) The Medium holder accommodates two 15 mL centrifuge or two 16x100 mm tubes (4 total)
c) The Large holder accommodates two 50 mL centrifuge tubes (4 total)
d) Two 200 mL tissue culture bottles directly without any tube holders.

(B) The SPHERO™ FlexiMag Separator Jr. (Cat. No. FMJ-1000) is designed for small scale use. It holds up to eight 1.5 mL microfuge tubes, 5 mL cryovials, 10x75 mm or 12x75mm tubes.

Magnetic Separator	Catalog No.	Unit
FlexiMag Magnetic Separator, Jr.	FMJ-1000	each
FlexiMag Magnetic Separator	FMS-1000	each
Tube Holder Set for FlexiMag Separator	MSS-1100	set
MiniTube Magnetic Separator	MTMS-16	each
HandiMag Magnetic Separator	HMS-1000	each
MicroMag Magnetic Separator	MMS-2100	each
UltraMag DW Separator (For Deep Well Plates)	UMDS-1000	each
UltraMag Magnetic Separator	UMS-3000	each

(C) The SPHERO™ MicroMag Separator (Cat No. MMS-2100) is designed to fit any 96-well plate with round bottom, flat bottom or V bottom. The magnet will pull the particles to the corner of the well bottom to facilitate the aspiration of supernatant during the washing.

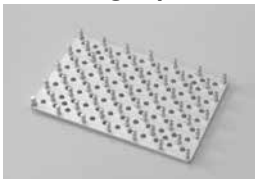
MicroMag Separator

(D) The SPHERO™ HandiMag Separator is a 1"x2"x0.375" Neodymium-Iron-Boron high strength magnet with nickel coating on the surface to prevent corrosion. It can be used to separate the magnetic particles in various containers such as microfuge tubes, test tubes or centrifuge tubes by holding the magnet against the wall of containers by hand or with a rubber band.

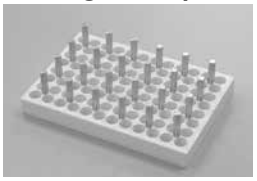
(E) The SPHERO™ UltraMag Separator (Cat. No. UMS-3000) and SPHERO™ UltraMag DW Separator (Cat. No. UMDS-1000) are designed to facilitate the washing of magnetic particles in the Magnetic Particles Enzyme Immunoassay (MPEIA) using 96-well plates. The magnetic pegs of UltraMag Separator fit between the wells underneath the 96-well plate. The microplate is read in an appropriate microtiter plate reader by loading the microplate with the attached UltraMag Separator into the reader. The magnets ensure that the magnetic particles stay out of the light beam passing through the bottom of the well and corresponding holes in the UltraMag Separator.

FlexiMag Separator Jr.

UltraMag Separator



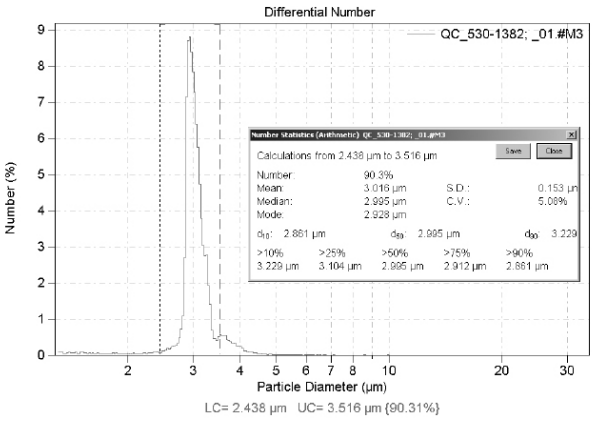
UltraMag DW Separator



SPHERO™ Coated Particles

- Manufactured by either passive adsorption or covalent coupling depending upon the intended application
- Stable for several years under proper storage condition
- Available in a wide variety of formats: polystyrene particles, fluorescent particles and magnetic particles coated with antibodies, Avidin, Streptavidin and Biotin and other proteins.

Figure 103 Size distribution analysis of SPHERO™ Cat. No. SVP-30-5, 3.0 µm Streptavidin Particles from the Beckman Coulter Multisizer™ 3.

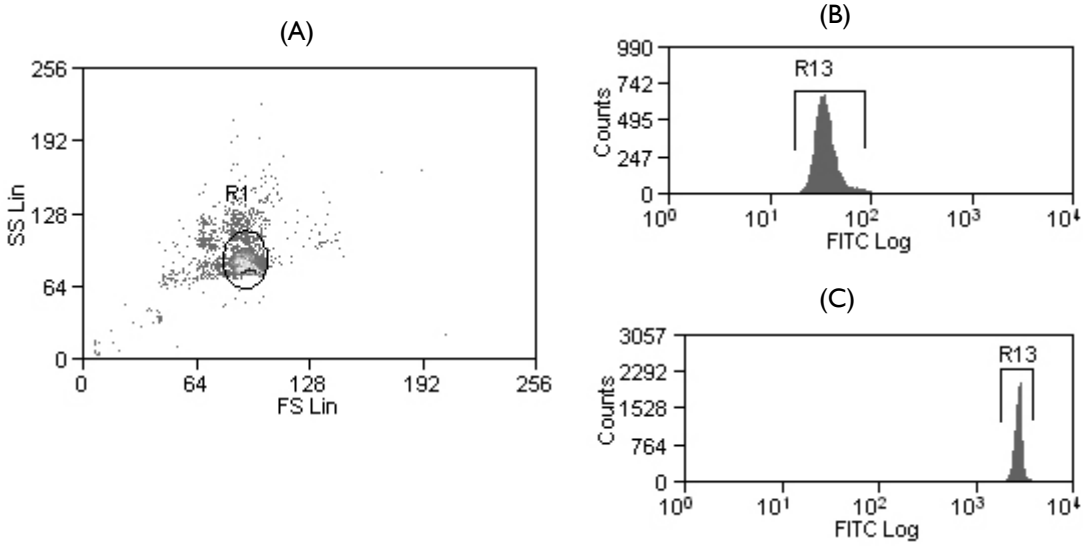


The SPHERO™ Coated Particles are prepared either by passive adsorption or covalent coupling, depending upon the intended applications. For example, the 4.0-4.5 µm Goat anti-Mouse IgG, Goat anti-Rabbit IgG and Goat anti-Human IgG coated magnetic particles intended for cell separation are prepared by covalent coupling. Similarly, the avidin and biotin coated particles are also prepared by covalent coupling. On the other hand, the 0.7-0.9 µm Goat anti-Mouse IgG coated polystyrene particles are prepared using passive adsorption. However, they are stable for several years under proper storage condition.

Spherotech offers a wide variety of polystyrene particles, fluorescent particles and magnetic particles coated with antibodies, Avidin, Streptavidin and Biotin and other proteins. For instance, Spherotech manufactures Protein A coated polystyrene and magnetic particles for binding to IgG from human, mouse and rabbit serum and Glutathione coated polystyrene particles for detecting the GST fusion proteins by flow cytometry. Likewise, Protease coated magnetic particles can be used for enzymatic digestion of antibodies or proteins.

Please refer to Page 90 or *www.Spherotech.com/tech* SpheroTechnical Note 1 for more detailed technical information regarding coating procedures.

Figure 104 (A) FSC vs SSC Histogram of SVP-60-5 (B)Histograms of SVP-60-5 before exposure to biotin-FITC (C) Histograms of SVP-60-5 after exposure to biotin-FITC.



SPHERO™ Streptavidin Coated Particles

- Designed for chemiluminescent assays, biotinylated ligand capture, molecular biology, immunoassay and sample prep applications
- Used to bind a variety of biotinylated ligands such as DNA/RNA, oligos, antibodies and proteins.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Streptavidin	0.05-0.1	0.1	SVP01-008-5	5 mL
Streptavidin	0.3-0.39	1.0	SVP-03-10	10 mL
Streptavidin	0.4-0.6	1.0	SVP-05-10	10 mL
Streptavidin	0.4-0.6	1.0	SVP-05-100	100 mL
Streptavidin	0.7-0.9	1.0	SVP-08-10	10 mL
Streptavidin	1.0-1.4	1.0	SVP-10-5	5 mL
Streptavidin	1.5-1.9	1.0	SVP-15-5	5 mL
Streptavidin	2.0-2.9	0.5	SVP-20-5	5 mL
Streptavidin	3.0-3.4	0.5	SVP-30-5	5 mL
Streptavidin	4.0-4.9	0.5	SVP-40-5	5 mL
Streptavidin	5.0-5.9	0.5	SVP-50-5	5 mL
Streptavidin	6.0-8.0	0.5	SVP-60-5	5 mL
Streptavidin	10.0-14.0	0.5	SVP-100-4	4 mL
Streptavidin	14.0-17.9	0.5	SVP-150-4	4 mL
Streptavidin	18.0-24.9	0.5	SVP-200-4	4 mL
Streptavidin	70.0-89.0	1.0	SVP-800-4	4 mL
Streptavidin	90.0-105.0	1.0	SVP-1000-4	4 mL
Streptavidin	196-211	1.0	SVP-2000-4	4 mL
Streptavidin, Blue	0.2-0.29	1.0	SVBP-02-10	10 mL
Streptavidin, Blue	0.3-0.39	1.0	SVBP-03-10	10 mL
Streptavidin, Cross-linked	0.7-0.9	1.0	SVPX-08-10	10 mL

Figure 105 Size distribution analysis of SPHERO™ Cat. No. SVP-05-10 (Streptavidin Polystyrene Particles, 1% w/v, 0.54 µm, 10 mL, Lot AC01)

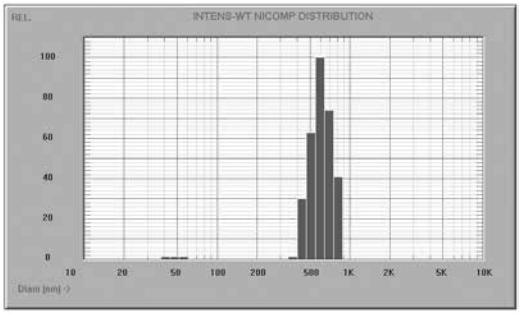
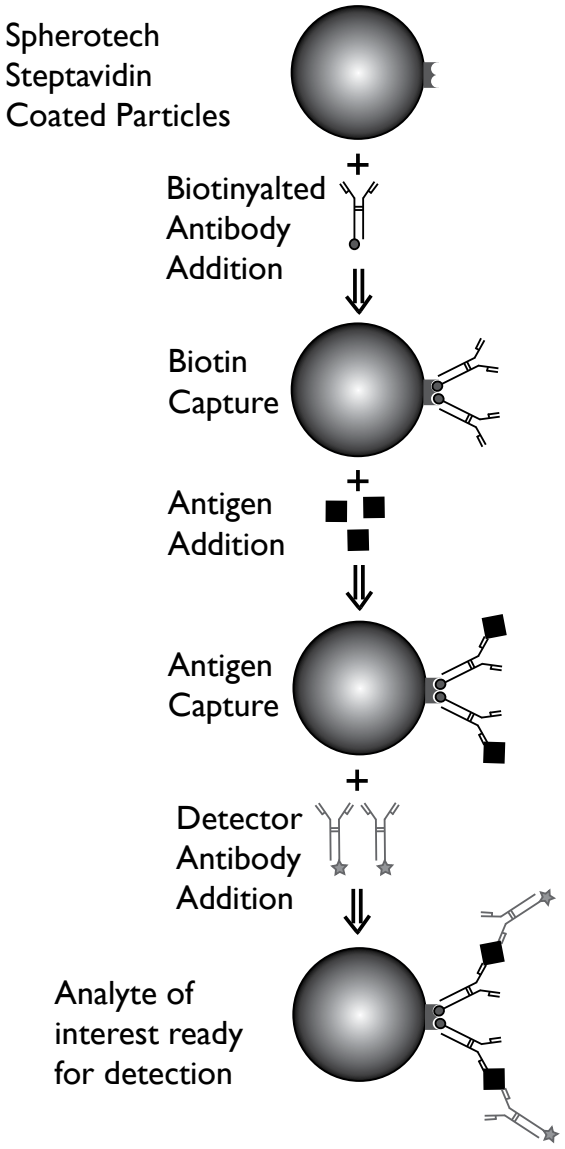


Figure 106 Example of streptavidin coated particles used in an sandwich immunoassay with a biotinylated antibody, antigen and labelled antibody.



SPHERO™ Avidin Coated Particles

- Prepared by covalent coupling of avidin to carboxyl polystyrene particles
- Contains two available biotin binding sites when coupled to particles
- Has a tendency to nonspecifically bind to other compounds due to high isoelectric point (pI), .

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Avidin	0.7-0.9	5.0	VP-08-10	10 mL
Avidin	1.0-1.9	5.0	VP-10-10	10 mL
Avidin	3.0-3.9	0.5	VP-30-5	5 mL
Avidin	6.0-8.0	0.5	VP-60-5	5 mL
Avidin, Cross-linked	6.0-8.0	0.5	VPX-60-5	5 mL
Avidin, Cross-linked	125-149	1.0	VPX-1400-4	4 mL

SPHERO™ Neutravidin Coated Particles

- Has a strong affinity for biotin
- Minimizes nonspecific adsorption due to neutral isoelectic point
- Has no known off-target binding domains like streptavidin.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Neutravidin	2.0-2.4	0.5	NVP-20-5	5 mL
Neutravidin	6.0-8.0	0.5	NVP-60-5	5 mL

SPHERO™ Biotin Coated Particles

- Has a strong affinity for avidin, streptavidin, and neutravidin tagged proteins
- Used when avidin, streptavidin or neutravidin is bound to proteins or oligonucleotides in immunoassay or molecular diagnostics
- Has been used to purify avidin from an intermediate product containing avidin and enzymes
- Reduces steric hindrances due to linker arms length used during coupling.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Iminobiotin	6.0-8.0	1.0	ITP-60-5	5 mL
Biotin	0.05-0.19	0.1	TP01-008-5	5 mL
Biotin	0.2-.3	0.1	TP01-025-5	5 mL
Biotin	0.7-0.9	1.0	TP-08-10	10 mL
Biotin	3.0-3.4	0.5	TP-30-5	5 mL
Biotin	6.0-8.0	1.0	TP-60-5	5 mL
Biotin, Cross-linked	6.0-8.0	1.0	TPX-60-5	5 mL
Biotin, Cross-linked	8.0-12.9	0.5	TPX-100-5	5 mL
Biotin, Cross-linked	13.0-17.9	0.5	TPX-150-5	5 mL

Figure 107 (A) FSC vs SSC Dot Plot and FSC Histogram of TP-30- 5 (Biotin Polystyrene Particles, 0.5% w/v, 3.27 um, 5 mL) Lot AG02.

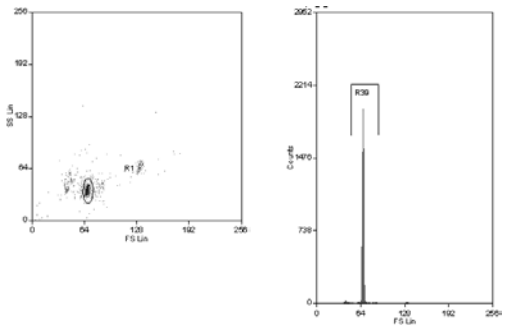
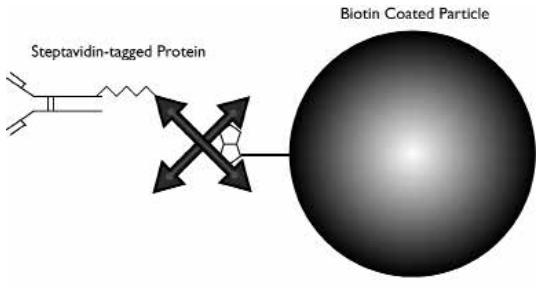


Figure 108 Example of biotin coated particles bound to streptavidin-tagged protein.

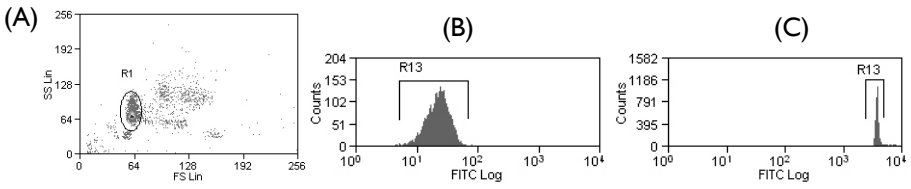


SPHERO™ Goat anti-Mouse IgG Coated Particles

- Prepared by the passive adsorption of polyclonal antibody to polystyrene particles
- Used for antibody attachment in immunoassay and cell separation applications
- The goat anti-Mouse IgG (H+L) coated particles are for general antibody attachment since they react with both the heavy and light chains of the IgG molecule (Fc and F(ab')2 / Fab portions)
- The goat anti-Mouse IgG Fc coated particles bind to the heavy chains of mouse IgG subclasses at the Fc region; thus, orientating the Fab fragments for optimal antigen binding.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Goat anti-Mouse IgG (H&L) , Blue	0.3-0.39	0.25	MPB-03-5	5 mL
Goat anti-Mouse IgG (H&L)	0.7-0.9	0.25	MP-08-20	20 mL
Goat anti-Mouse IgG (H&L)	0.7-0.9	0.25	MP-08-100	100 mL
Goat anti-Mouse IgG (H&L)	6.0-8.0	0.5	MP-60-5	5 mL
Goat anti-Mouse IgG (H&L) , Cross adsorbed	0.7-0.9	0.25	MPXA-08-20	20 mL
Goat anti-Mouse IgG (H&L), Cross adsorbed	0.7-0.9	0.25	MPXA-08-100	100 mL
Goat anti-Mouse IgG (H&L), Cross adsorbed	6.0-8.0	0.5	MPXA-60-5	5 mL
Goat anti-Mouse IgG (Fc)	0.7-0.9	0.25	MPFc-08-20	20 mL
Goat anti-Mouse IgG (Fc)	0.7-0.9	0.25	MPFc-08-100	100 mL
Goat anti-Mouse IgG (Fc)	3.0-3.9	0.5	MPFc-30-5	5 mL
Goat anti-Mouse IgG (Fc)	6.0-8.0	0.5	MPFc-60-5	5 mL
Goat anti-Mouse IgG (Fc)	13.0-17.9	0.5	MPFc-150-4	4 mL

Figure 109 (A) FSC vs SSC Histogram of MPFc-60-5 (B)Histograms of MPFc-60-5 before exposure to biotin-FITC (C) Histograms of MPFc-60-5 after exposure to biotin-FITC.



SPHERO™ Goat anti-Rat IgG Coated Particles

- Coated with affinity-purified polyclonal antibodies with specificity for rat immunoglobulin classes (IgG, IgM)
- The antibodies used are purified to minimize cross-reactivity to serum proteins of other species
- Cat. No. RtPFc-60-5 reacts with rat IgG heavy chains antibody portions in immunoassay and cell separation applications
- Cat. No. RtPXA-60-5 reacts with rat IgG heavy and light chains antibody portions.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Goat anti-Rat IgG (Fc), Cross adsorbed	6.0-8.0	0.5	RtPFc-60-5	5 mL
Goat anti-Rat IgG (H&L), Cross adsorbed	6.0-8.0	0.5	RtPXA-60-5	5 mL

SPHERO™ Goat anti-Human IgG Coated Particles

- Reacts with whole molecule human IgG and with the light chains of other human immunoglobulins
- Used as Human IgG Capture beads for Flow Cytometry Bead Based Assays.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Goat anti-Human IgG (H&L)	0.7-0.9	0.25	HUP-08-5	5 mL
Goat anti-Human IgG (H&L)	3.0-3.4	0.5	HUP-30-5	5 mL
Goat anti-Human IgG (H&L)	6.0-8.0	0.5	HUP-60-5	5 mL
Goat anti-Human IgG (H&L)	10.0-14.0	0.5	HUP-100-5	5 mL
Goat anti-Human IgG (H&L)	14.0-17.9	0.5	HUP-150-5	5 mL
Goat anti-Human IgG (Fc), Blue	3.0-3.9	0.5	HPBFc-30-5	5 mL

SPHERO™ Donkey anti-Goat IgG Coated Particles

- Used in flow cytometric assays and fluorometric microvolume assays (FMAT®, Applied Biosystems, Foster City, CA)
- Reacts with the heavy and light chains of Goat IgG and may also react with the light chains of other goat immunoglobulins.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Donkey anti-Goat IgG (H&L), Cross-adsorbed	6.0-8.0	0.5	GPXA-60-5	5 mL

SPHERO™ Goat anti-Rabbit IgG Coated Particles

- Diameter of 0.8 µm used in bead uptake experiment to study focal exocytosis and phagocytosis in macrophages
- Gt anti-Rb beads with a diameter of 7.4 µm are used in flow cytometric assays and fluorometric microvolume assays (FMAT®, Applied Biosystems, Foster City, CA).

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Goat anti-Rabbit IgG (Fc)	0.7-0.9	0.25	RPFc-08-20	20 mL
Goat anti-Rabbit IgG (Fc)	0.7-0.9	0.25	RPFc-08-100	100 mL
Goat anti-Rabbit IgG (Fc)	6.0-8.0	0.5	RPFc-60-5	5 mL

SPHERO™ Donkey anti-Sheep IgG Coated Particles

- Uses antibodies cross reacted to chicken, guinea pig, hamster, horse, human, mouse, and rat serum proteins.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Donkey anti-Sheep IgG (H&L), Cross adsorbed	6.0-8.0	0.5	SPXA-60-5	5 mL

SPHERO™ Mouse IgG Coated Particles

- Prepared by covalently coupling of whole molecule Mouse IgG
- Binds to ~4.53 µg of Mouse IgG-FITC / mg of particles.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Mouse IgG, Cross linked	5.0-5.9	0.5	MsGPX-50-5	5 mL

SPHERO™ Protein A Coated Particles

- Produced by covalently coupling Protein A to polystyrene particles
- Interacts with the Fc region of IgGs of several species (see Table 2 below).

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Protein A	0.7-0.9	1.0	PAP-08-5	5 mL
Protein A	2.0-2.9	0.5	PAP-20-5	5 mL
Protein A	4.0-4.9	0.5	PAP-40-5	5 mL
Protein A	6.0-8.0	0.5	PAP-60-5	5 mL

SPHERO™ Protein G Coated Particles

- Produced by covalently coupling Protein G to polystyrene particles
- Has a high specificity for the Fc regions of IgGs of different species (see Table 3 below)
- Suitable for easy and efficient one-step affinity purification of small amounts of Ig and other proteins.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Protein G	0.4-0.6	1.0	PGP-05-5	5 mL
Protein G	0.7-0.9	1.0	PGP-08-5	5 mL
Protein G	2.0-2.9	0.5	PGP-20-5	5 mL
Protein G	3.0-3.4	0.5	PGP-30-5	5 mL
Protein G	4.0-4.9	0.5	PGP-40-5	5 mL
Protein G	6.0-8.0	0.5	PGP-60-5	5 mL
Protein G	13.0-17.9	0.5	PGP-150-4	4 mL

Table 2 Binding strength of Protein A to immunoglobulins from different species.

Ig origin	Binding to Protein A
Human IgG1, IgG2 and IgG4	Strong
Human IgG3, IgA and IgM	Weak
Human IgD	No binding
Mouse IgG2a, IgG2b and IgG3	Strong
Mouse IgG1	Weak
Mouse IgM	No binding
Rat IgG1	Weak
Rat IgG2a and IgG2b	No binding
Rat IgG2c	Stong
Bovine IgG1	Weak
Bovine IgG2	Strong
Chicken IgY	No binding
Dog IgG	Strong
Goat IgG1 and IgG2	Strong
Guinea pig IgG	Strong
Horse IgG	No binding
Monkey IgG	Strong
Porcine IgG	Strong
Rabbit IgG	Strong
Sheep IgG1	Weak
Sheep IgG2	Strong

Table 3 Binding strength of Protein G to immunoglobulins from different species.

Ig origin	Binding to Protein G
Human IgG1, IgG2, IgG3 and IgG4	Strong
Human IgA, IgD, IgE and IgM	No binding
Mouse IgG1, IgG2a, IgG2b and IgG3	Strong
Mouse IgM	Weak
Rat IgG1and IgG2b	Weak
Rat IgG2a and IgG2c	Strong
Bovine IgG	Strong
Chicken IgY	No binding
Dog IgG	Weak
Goat IgG1 and IgG2	Strong
Guinea pig IgG	Weak
Horse IgG	Strong
Monkey IgG	Strong
Porcine IgG	Strong
Rabbit IgG	Strong
Sheep IgG1 and IgG2	Strong

SPHERO™ Anti-DNP Coated Particles

- Prepared by covalently coupling Rat anti-Dinitrophenol to carboxyl polystyrene particles using EDC
- Has a high affinity for the dinitrophenyl (DNP) hapten
- Binds to DNP-labeled molecules including nucleic acid probes.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Anti-DNP	5.0-5.9	0.1	DNPP-50-2	2 mL

SPHERO™ Anti-His Coated Particles

- Prepared by covalently coupling anti-6X His EPITOPE TAG (Rabbit) to carboxyl polystyrene particles using EDC
- Has high affinity to His-tag fusion proteins
- Simplifies the purification and detection of recombinant protein fused with 6X-His tag.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Rabbit anti-6X Histidine	0.4-0.6	0.1	HISP-05-2	2 mL
Rabbit anti-6X Histidine	6.0-8.0	0.1	HISP-60-2	2 mL

SPHERO™ BSA Coated Particles

- Used as a control during the determination of antibody nonspecific binding
- Has several functional groups available for conjugation.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
BSA	0.7-0.9	1.0	BP-08-10	10 mL
BSA	3.0-3.9	1.0	BP-30-5	5 mL
BSA	6.0-8.0	1.0	BP-60-5	5 mL
BSA	13.0-17.9	2.0	BP2-150-5	5 mL

SPHERO™ Anti-Digoxigenin Coated Particles

- Prepared by covalently coupling of monoclonal antibody to digoxigenin from mouse-mouse hybrid cells
- Used to purify and detect digoxigenin-labeled protein and nucleic acids.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Anti-Digoxigenin	2.0-2.4	0.1	DIGP-20-2	2 mL
Anti-Digoxigenin	4.0-4.9	0.1	DIGP-40-2	2 mL

SPHERO™ Glutathione Coated Particles

- Prepared by covalently coupling
- Used to purify and detect glutathione-s-transferase (GST) fusion proteins.

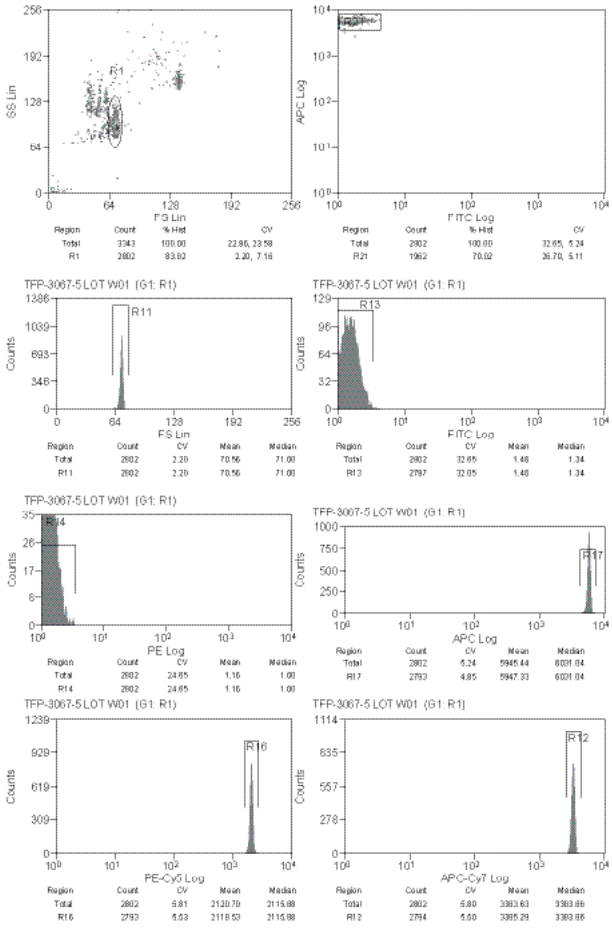
Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Glutathione	2.0-2.9	0.5	GSHP-20-5	5 mL
Glutathione	4.0-4.9	0.5	GSHP-40-5	5 mL
Glutathione	6.0-8.0	0.5	GSHP-60-5	5 mL

SPHERO™ Coated Fluorescent Particles

Spherotech offers a wide variety of fluorescent particles coated with antibodies, Avidin, Biotin, Protein A and Protein G for the convenience of our customers. Please refer to Page 80 for more detailed technical information and coating procedures. In addition, please see the Fluorescent Particle Page 14 for the excitation and emission spectra of the fluorophores used to produce the SPHERO™ Coated Fluorescent Microparticles.

SPHERO™ Biotin Coated Fluorescent Particles

Figure 110 Histograms of TFP-3067-5 (Biotin Fluorescent Beads, Blue, 0.1% w/v, 3.6 µm, 5 mL).



- Biotinylated particles exhibits high affinity noncovalent interaction with streptavidin
- Blue Fluorescent Biotin Particles may be used in Flow Cytometry Bead Assays since they exhibit limited FITC and PE fluorescence, but are fluorescent in PE-Cy5, APC, and APC-Cy7 channels
- Biotin beads with a 0.5µm diameter have been used in FACS phagocytosis assay and immunofluorescence procedure in macrophages.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Biotin, Fluorescent ,Yellow	0.05-0.15	0.1	TFP-00852-5	5 mL
Biotin, Fluorescent ,Yellow	0.4-0.6	0.1	TFP-0552-5	5 mL
Biotin, Fluorescent, Nile Red	0.4-0.6	0.1	TFP-0556-5	5 mL
Biotin, Fluorescent, Pink	0.7-0.9	0.1	TFP-0858-5	5 mL
Biotin, Fluorescent, Pink	1.7-2.2	0.1	TFP-2058-5	5 mL
Biotin, Fluorescent, Blue	3.0-3.9	0.1	TFP-3067-5	5 mL
Biotin, Fluorescent, Pink	5.0-5.9	0.1	TFP-5058-5	5 mL
Biotin, Fluorescent, Blue	5.0-5.9	0.1	TFP-5067-5	5 mL
Biotin, Fluorescent, Yellow	7.0-7.9	0.1	TFP-7052-5	5 mL
Biotin, Fluorescent, Nile Red	7.0-7.9	0.1	TFP-7056-5	5 mL
Biotin, Fluorescent, Blue	7.0-7.9	0.1	TFP-7067-5	5 mL

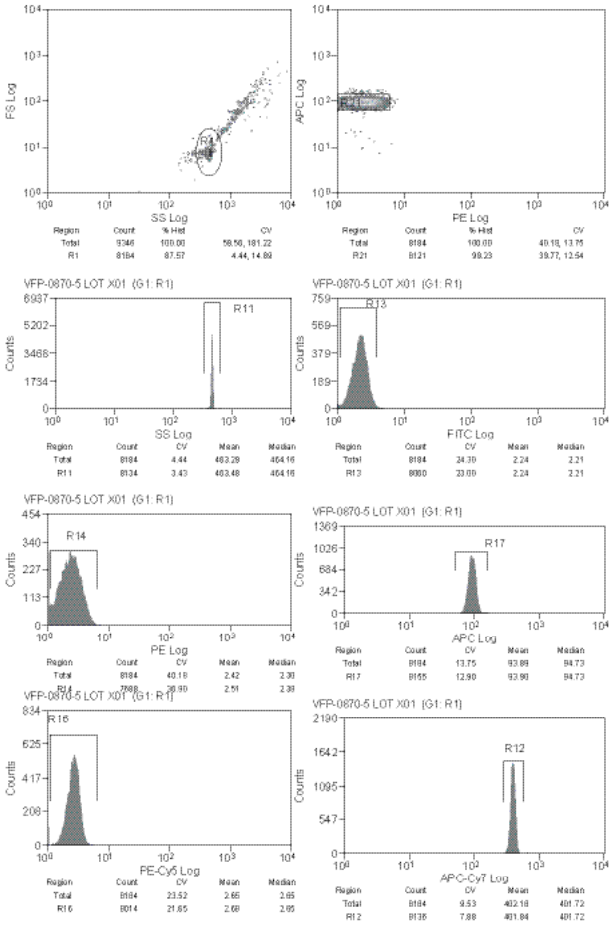
SPHERO™ Glutathione Coated Fluorescent Particles

- Prepared by covalently coupling
- Used to purify and detect glutathione-s-transferase (GST) fusion proteins.

Particle Type and Surface	Size, µm	Catalog No.	Unit
Glutathione, Fluorescent, PAK Blue, 10^7/mL	10.0-14.0	GSHFP-10067-2	2 mL

SPHERO™ Avidin Coated Fluorescent Particles

Figure 111 Histograms of VFP-0870-5, Lot No. X01(Avidin Fluorescent Particles, Sky Blue, 0.1% w/v, 0.88µm, 5 mL).



- Strong and specific affinity for Biotin
- Extensive chemical modification during coupling to beads has little effect on activity, making Avidin specifically useful for detection and purification of proteins.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Avidin, Fluorescent, Yellow	0.4-0.6	0.1	VFP-0552-5	5 mL
Avidin, Fluorescent, Pink	0.4-0.6	0.1	VFP-0558-5	5 mL
Avidin, Fluorescent, Purple	0.4-0.6	0.1	VFP-0562-5	5 mL
Avidin, Fluorescent, Yellow	0.7-0.9	0.1	VFP-0852-5	5 mL
Avidin, Fluorescent, Nile Red	0.7-0.9	0.1	VFP-0856-5	5 mL
Avidin, Fluorescent, Purple	0.7-0.9	0.1	VFP-0862-5	5 mL
Avidin, Fluorescent, Sky Blue	0.7-0.9	0.1	VFP-0870-5	5 mL
Avidin, Fluorescent, Yellow	1.7-2.2	0.1	VFP-2052-5	5 mL
Avidin, Fluorescent, Pink	1.7-2.2	0.1	VFP-2058-5	5 mL

SPHERO™ Streptavidin Coated Fluorescent Particles

- Streptavidin fluorescent particles provide a universal binding reagent that simplifies clinical diagnostics, immuno/histological studies and research applications
- Streptavidin fluorescent beads with a diameter of 0.5µm immobilized with fusion proteins have been used to quantitatively analyze low-affinity interactions at the cell surface by determining its binding to cells using flow cytometry.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Streptavidin, Fluorescent Yellow	0.4-0.6	0.1	SVFP-0552-5	5 mL
Streptavidin, Fluorescent Nile Red	0.4-0.6	0.1	SVFP-0556-5	5 mL
Streptavidin, Fluorescent Blue	1.0-1.9	0.1	SVFP-1068-5	5 mL
Streptavidin, Fluorescent Nile Red	5.0-7.9	0.1	SVFP-6056-5	5 mL

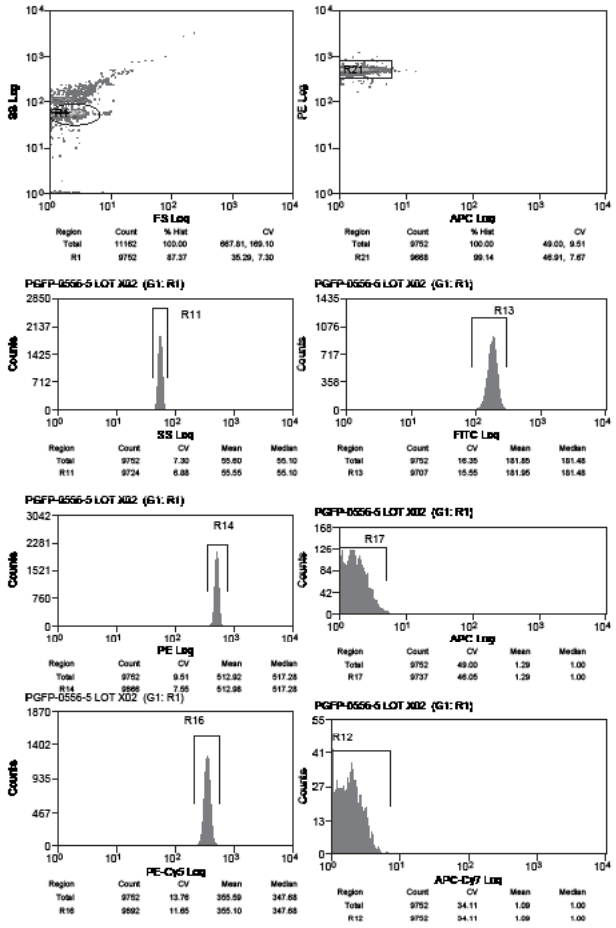
SPHERO™ Protein A Coated Fluorescent Particles

- Interacts with the Fc region of IgGs of several species - (see Table 2 on Page 66)
- See Page 14 for the excitation/emission spectra of the fluorophores used by Spherotech.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Protein A, Fluorescent Yellow	0.4-0.6	0.1	PAFP-0552-5	5 mL
Protein A, Fluorescent Nile Red	0.4-0.6	0.1	PAFP-0556-5	5 mL
Protein A, Fluorescent Pink	0.4-0.6	0.1	PAFP-0558-5	5 mL
Protein A, Fluorescent Purple	0.4-0.6	0.1	PAFP-0562-5	5 mL
Protein A, Fluorescent Yellow	38.0-44.0	1.0	PAFP-40052-5	5 mL

SPHERO™ Protein G Coated Fluorescent Particles

Figure 112 Histograms of PGFP-0556-5, Lot No. Y01 (Protein G Fluorescent Particles, Nile Red, 0.1% w/v, 0.54 µm, 5 mL).



- Powerful tool for binding and detecting both monoclonal and polyclonal antibodies
- Protein G is a bacterial cell wall protein isolated from group G streptococci
- Binds to IgG with Fc region specificity.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Protein G, Fluorescent, Yellow	0.4-0.6	0.1	PGFP-0552-5	5 mL
Protein G, Fluorescent, Nile Red	0.4-0.6	0.1	PGFP-0556-5	5 mL
Protein G, Fluorescent, Pink	0.4-0.6	0.1	PGFP-0558-5	5 mL
Protein G, Fluorescent, Purple	0.4-0.6	0.1	PGFP-0562-5	5 mL
Protein G, Fluorescent, Pink	5.0-5.9	0.1	PGFP-5058-5	5 mL
Protein G, Fluorescent, Yellow	38.0-44.0	1.0	PGFP-40052-5	5 mL

SPHERO™ Protein A/G Coated Fluorescent Particles

- Prepared by covalently coupling
- Convenience of IgG binding domains of both Protein A and Protein G on one bead.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Protein A/G, Fluorescent, Yellow	38.0-44.0	1.0	PAGFP-40052-5	5 mL

SPHERO™ Goat anti-Mouse IgG Coated Fluorescent Particles

- Offering both Goat anti-Mouse IgG H&L and Fcγ Fragment Specific coated fluorescent particles
- IgG Fc Fragment Specific antibody reacts with Fc portion of Mouse IgG heavy chains but not with the Fab portion
- Available in a wide variety of sizes and fluorophores
- Used in FACS based cell-binding analysis method for detecting protein-protein interaction
- Used in phagocytosis studies where the number of internalized microspheres per cell was determined from flow cytometry fluorescence histograms.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Goat anti-Mouse IgG (Fc) Fluorescent ,Yellow	2.5-4.5	0.1	MFcP-3052-5	5 mL
Goat anti-Mouse IgG (Fc) Fluorescent, Nile Red	2.5-4.5	0.1	MFcP-3056-5	5 mL
Goat anti-Mouse IgG (Fc) Fluorescent, Pink	2.5-4.5	0.1	MFcP-3058-5	5 mL
Goat anti-Mouse IgG (Fc) Fluorescent, Blue	2.5-4.5	0.1	MFcP-3068-5	5 mL
Goat anti-Mouse IgG (Fc) Fluorescent, Ocean Blue	2.5-4.5	0.1	MFcP-3069-5	5 mL
Goat anti-Mouse IgG (H&L) Fluorescent ,Yellow	0.4-0.6	0.1	MFP-0552-5	5 mL
Goat anti-Mouse IgG (H&L) Fluorescent, Nile Red	0.4-0.6	0.1	MFP-0556-5	5 mL
Goat anti-Mouse IgG (H&L) Fluorescent, Purple	0.4-0.6	0.1	MFP-0562-5	5 mL
Goat anti-Mouse IgG (H&L) Fluorescent, Yellow	0.7-0.9	0.1	MFP-0852-5	5 mL
Goat anti-Mouse IgG (H&L) Fluorescent, Nile Red	0.7-0.9	0.1	MFP-0856-5	5 mL
Goat anti-Mouse IgG (H&L) Fluorescent, Pink	0.7-0.9	0.1	MFP-0858-5	5 mL
Goat anti-Mouse IgG (H&L) Fluorescent, Purple	0.7-0.9	0.1	MFP-0862-5	5 mL
Goat anti-Mouse IgG (H&L) Fluorescent, Yellow	1.7-2.2	0.1	MFP-2052-5	5 mL
Goat anti-Mouse IgG (H&L) Fluorescent, Nile Red	1.7-2.2	0.1	MFP-2056-5	5 mL
Goat anti-Mouse IgG (H&L) Fluorescent, Pink	1.7-2.2	0.1	MFP-2058-5	5 mL
Goat anti-Mouse IgG (H&L) Fluorescent, Purple	1.7-2.2	0.1	MFP-2062-5	5 mL
Goat anti-Mouse IgG (H&L) Fluorescent, Sky Blue	1.7-2.2	0.1	MFP-2070-5	5 mL

SPHERO™ Goat anti-Human IgG Coated Fluorescent Particles

- Reacts with whole molecule Human IgG
- Reacts with light chains of other human immunoglobulins.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Goat anti-Human IgG (H&L), Fluorescent ,Yellow	0.7-0.9	0.1	HFP-0852-5	5 mL
Goat anti-Human IgG (H&L), Fluorescent, Nile Red	0.7-0.9	0.1	HFP-0856-5	5 mL
Goat anti-Human IgG (H&L), Fluorescent, Purple	0.7-0.9	0.1	HFP-0862-5	5 mL

SPHERO™ Rat anti-Mouse IgM Coated Fluorescent Particles

- Coated with Rat monoclonal anti-Mouse Immunoglobulin IgM by covalent coupling
- Reacts with Mouse mu heavy chain of immunoglobulin.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Rat anti-Mouse IgM Fluorescent, Nile Red	0.7-0.9	0.1	MFPIgM-0856-5	5 mL

SPHERO™ Coated Magnetic Particles

- Available with a variety of ligands such as Streptavidin, Avidin, Neutravidin, Protein A, Protein G, and Biotin
- Also available coated with highly specific recognition groups such as polyclonal antibodies
- Used in nucleic acid isolation, protein purification, immunology, and cell separations
- Available impregnated with fluorophores for flow cytometry or easy particle location identification in phagocytosis assays.

Magnetic particles coated with Avidin, Streptavidin, Biotin, Protein A and various antibodies are available from Spherotech. All of the proteins used are covalently coupled to the magnetic particles. The coated magnetic particles are supplied as a suspension in phosphate buffer, pH 7.4 with 0.02% sodium azide (some products also contain 0.1% BSA). Please refer to the recommended coating procedures on page 88 for more detailed technical information and coating.

Similarly to the magnetic particles offered on page 61 Spherotech coated magnetic particles are offered as the classic, encapsulated, or crosslinked magnetic microsphere. See pages 62 for benefits of each type.

SPHERO™ Biotin Coated Magnetic Particles

- Used to take advantage of the high affinities of the biotin–streptavidin and biotin–avidin interactions (Ka in the order of 10^{13}–10^{15} M^{-1})
- Represents one of the strongest biomolecules interactions to form stable complexes.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Biotin	1.0-1.4	0.5	TM-10-10	10 mL
Biotin	4.0-4.5	1.0	TM-40-10	10 mL
Biotin	6.0-7.9	1.0	TM-60-5	5 mL
Biotin, Cross-linked, granules, non-uniform	~1-2 µm	0.5	TMX-10-10	10 mL

SPHERO™ Avidin Coated Magnetic Particles

- Used for Genome isolation when coated with a biotinylated genome capture probe for E.coli and B.subtilis*

  *S. Yeung, T. Ming-Hung Lee, H. Cai, and I-Ming Hsing. "A DNA biochip for on-the-spot multiplexed pathogen identification." Nucleic Acids Res., Vol 34, No. 18, e118 (Oct 2006)

- See pages 70 and 71 for uses of streptavidin and avidin coated particles.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Avidin	1.0-1.4	0.5	VM-10-10	10 mL
Avidin	4.0-4.5	1.0	VM-40-10	10 mL
Avidin	4.0-4.5	1.0	VM-40-100	100 mL
Avidin	6.0-8.0	1.0	VM-60-10	10 mL
Avidin	6.0-8.0	1.0	VM-60-100	100 mL
Avidin	8.1-9.9	1.0	VM-80-5	5 mL
Avidin, Smooth Surface	3.0-3.9	1.0	VMS-30-10	10 mL
Avidin, Smooth Surface	4.0-5.0	1.0	VMS-40-10	10 mL
Avidin, Cross-linked, granules, non-uniform	~1-2 µm	0.5	VMX-10-10	10 mL
Avidin, Cross-linked, granules, non-uniform	~1-2 µm	0.5	VMX-10-100	100 mL

SPHERO™ Streptavidin Coated Magnetic Particles

- Streptavidin magnetic particles have found widespread use as detection reagents in immunology, biochemistry and cell biology due to their high affinity binding to biotin
- Biotin–streptavidin interaction have been exploited in many applications including the development of new reagents for diagnostics such as sandwich magnetic particle enzyme-linked immunosorbent assay (MPEIA) and molecular biology studies involving nucleic acids.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Streptavidin, High Iron	0.2-0.39	0.5	SVM-025-5H	5 mL
Streptavidin	0.4-0.69	0.5	SVM-05-10	10 mL
Streptavidin, High Iron	0.4-0.69	0.5	SVM-05-5H	5 mL
Streptavidin	0.7-0.9	0.5	SVM-08-10	10 mL
Streptavidin	1.0-1.4	0.5	SVM-10-10	10 mL
Streptavidin	1.5-1.9	0.5	SVM-15-10	10 mL
Streptavidin	2.0-2.9	0.5	SVM-20-10	10 mL
Streptavidin	3.0-3.9	1.0	SVM-30-10	10 mL
Streptavidin	4.0-4.5	1.0	SVM-40-10	10 mL
Streptavidin	4.6-5.9	1.0	SVM-50-5	5 mL
Streptavidin	6.0-7.9	1.0	SVM-60-5	5 mL
Streptavidin	8.0-9.9	1.0	SVM-80-5	5 mL
Streptavidin, High Iron	38.0-44.0	0.5	SVMH-400-4	4 mL
Streptavidin, High Iron	45.0-52.0	0.5	SVMH-500-4	4 mL
Streptavidin, Smooth Surface	3.0-3.9	1.0	SVMS-30-10	10 mL
Streptavidin, Smooth Surface	4.0-5.0	1.0	SVMS-40-10	10 mL
Streptavidin, Cross-linked, granules, non-uniform	~1-2 µm	0.5	SVMX-10-10	10 mL

SPHERO™ Neutravidin Coated Magnetic Particles

- Provide very low non-specific binding since they do not contain any carbohydrates and have a near-neutral isoelectric point of 6.3
- Used for diagnostic and molecular biology applications
- Have a binding capacity of ~5100 pmol/mg.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Neutravidin	2.0-2.9	1.0	NVM-20-5	5 mL

SPHERO™ Rabbit anti-HA Coated Magnetic Particles

- Coated with affinity purified anti-Hemagglutinin (HA) epitope tag [Rabbit] polycolonal antibody
- Binds to HA-tagged recombinant proteins.

Particle Type and Surface	Size, µm	Catalog No.	Unit
Rabbit anti-HA, Smooth Surface, 10^7/mL	3.0-3.9	RHAMS-30-2	2 mL

SPHERO™ Protein G Coated Magnetic Particles

- Used to link capture species-specific anti-IgG to magnetic microspheres
- Directly binds immunoglobulins from ascites fluids or concentrated hybridoma supernatants to facilitate purification.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Protein G	4.0-4.5	1.0	PGM-40-5	5 mL
Protein G, Smooth Surface	3.0-3.9	1.0	PGMS-30-5	5 mL
Protein G, Smooth Surface	4.0-5.0	1.0	PGMS-40-5	5 mL
Protein G, Cross-linked, granules, non-uniform	~1-2	1.0	PGMX-10-5	5 mL

SPHERO™ Protein A Coated Magnetic Particles

- Used for the immunomagnetic separation (IMS) and real-time PCR to detect Escherichia coli*

*Fu, Z., S. Rogelj, et al. (2005). "Rapid detection of Escherichia coli O157:H7 by immunomagnetic separation and real-time PCR." International Journal of Food Microbiology 99(1): 47-57.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Protein A	4.0-4.5	1.0	PAM-40-5	5 mL
Protein A, Smooth Surface	3.0-3.9	1.0	PAMS-30-5	5 mL
Protein A, Smooth Surface	4.0-5.0	1.0	PAMS-40-5	5 mL
Protein A, Cross-linked, granules, non-uniform	~1-2	1.0	PAMX-10-5	5 mL

SPHERO™ Sheep anti-Rat IgG Coated Magnetic Particles

- Consists of uniform, paramagnetic polystyrene beads coated with polyclonal Sheep anti-Rat IgG antibodies.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Sheep anti-Rat IgG (H&L)	4.0-4.5	2.0	SRM-40-5	5 mL

SPHERO™ Donkey anti-Goat IgG Coated Magnetic Particles

- Ideal for direct or indirect isolation of proteins and cells during immunomagnetic separation.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Donkey anti-Goat IgG (H&L) Cross-adsorbed	4.0-4.5	1.0	GMXA-40-10	10 mL

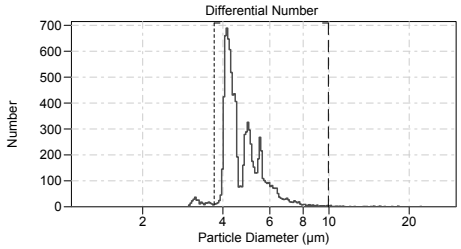
SPHERO™ Goat anti-Rabbit IgG Coated Magnetic Particles

- Used in immunomagnetic separation (IMS)*

* Antognoli, M. C., M. D. Salman, et al. (2001). "A one-tube nested polymerase chain reaction for the detection of mycobacterium bovis in spiked milk samples: an evaluation of concentration and lytic techniques." J Vet Diagn Invest 13(2): 111-116.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Goat anti-Rabbit IgG (H&L)	4.0-4.9	1.0	RM-40-10	10 mL
Goat anti-Rabbit IgG (Fc)	4.0-4.5	1.0	RMFc-40-10	10 mL
Goat anti-Rabbit IgG (H&L), Smooth Surface	3.0-3.9	1.0	RMS-30-10	10 mL
Goat anti-Rabbit IgG (H&L), Smooth Surface	4.0-5.0	1.0	RMS-40-10	10 mL
Goat anti-Rabbit IgG (Fc), Smooth Surface	3.0-3.9	1.0	RMSFc-30-10	10 mL
Goat anti-Rabbit IgG (H&L), Cross-linked, granules, non-uniform	~1-2 µm	0.5	RMX-10-10	10 mL

Figure 113 Size distribution analysis of SPHERO™ Cat. No. RM-40-10, Gt anti-Rb IgG (H&L) Magnetic Particles from the Beckman Coulter Z3 Multisizer™.



SPHERO™ Goat anti-Mouse IgG Coated Magnetic Particles

Attributes

- Uniform particle size
- Paramagnetic in nature
- Rapid magnetic responsiveness
- Low non-specific binding
- High binding capacity
- Consistent lot-to-lot performance.

Applications

- Automated immunoassays
- Immunoprecipitation
- IP-western blots.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Goat anti-Mouse IgG (H&L)	4.0-4.5	1.0	MM-40-10	10 mL
Goat anti-Mouse IgG (Fc)	4.0-4.5	1.0	MMFc-40-10	10 mL
Goat anti-Mouse IgG (H&L) , Cross adsorbed	4.0-4.5	1.0	MMXA-40-10	10 mL
Goat anti-Mouse IgG (H&L), Smooth Surface	3.0-3.9	1.0	MMS-30-10	10 mL
Goat anti-Mouse IgG (H&L), Smooth Surface	4.0-5.0	1.0	MMS-40-10	10 mL
Goat anti-Mouse IgG (Fc), Smooth Surface	3.0-3.9	1.0	MMSFc-30-10	10 mL
Goat anti-Mouse IgG (Fc), Smooth Surface	4.0-5.0	1.0	MMSFc-40-10	10 mL
Goat anti-Mouse IgG (H&L), Smooth Surface, Cross adsorbed	3.0-3.9	1.0	MMSXA-30-10	10 mL
Goat anti-Mouse IgG (H&L), Smooth Surface, Cross adsorbed	4.0-5.0	1.0	MMSXA-40-10	10 mL
Goat anti-Mouse IgG (H&L), Cross-linked, granules, non-uniform	~1-2 µm	0.5	MMX-10-10	10 mL
Goat anti-Mouse IgG (H&L), Cross adsorbed, Cross-linked, granules, non-uniform	~1-2 µm	0.5	MMXA-10-10	10 mL

SPHERO™ Goat anti-Human IgG Coated Magnetic Particles

- Ideal for the capture and/or detection of target analytes by direct or indirect isolation during immunomagnetic separation
- Improves the performance of ELISAs by enhancing sensitivity and shortening incubation times.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Goat anti-Human IgG (H&L)	4.0-4.5	1.0	HM-40-10	10 mL
Goat anti-Human IgG (H&L) , Smooth Surface	3.0-3.9	1.0	HMS-30-10	10 mL
Goat anti-Human IgG (H&L) , Smooth Surface	4.0-5.0	1.0	HMS-40-10	10 mL
Goat anti-Human IgG (H&L), Cross-linked, granules, non-uniform	~1-2 µm	0.5	HMX-10-10	10 mL

SPHERO™ Con A Coated Magnetic Particles

- Binds to saccharide functional groups on the cell surface*

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Con A	0.7-0.9	1.0	CAM-08-10	10 mL

*Gupta, S., R. G. Alargova, et al. "On-Chip Dielectrophoretic Coassembly of Live Cells and Particles into Responsive Biomaterials." Langmuir, 2010, 26 (5), pp 3441–3452.

SPHERO™ Coated Magnetic Fluorescent Particles

SPHERO™ Biotin Coated Magnetic Fluorescent Particles

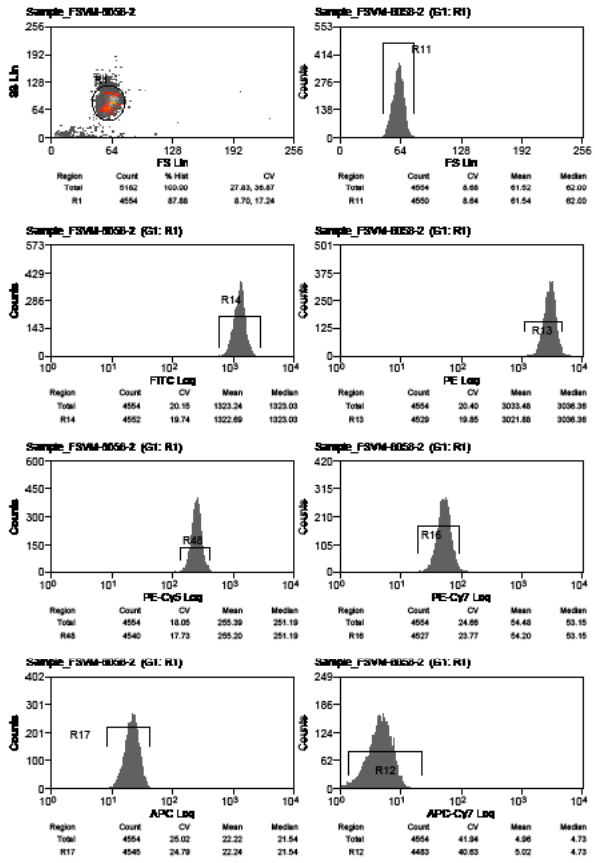
- Offering both Biotin and Streptavidin coated paramagnetic fluorescent particles.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Biotin, Fluorescent Nile Red	4.0-4.9	0.1	TFM-4056-5	5 mL

- Reference Page 88 for more detailed technical information and coating procedures.
- Reference Fluorescent Particle Page 14 for the excitation and emission spectra of the fluorophores used to produce the SPHERO™ Coated Magnetic Fluorescent Microparticles.

SPHERO™ Streptavidin Coated Magnetic Fluorescent Particles

Figure 114 Histograms of FSVM-8058-2, No. Lot Z01 (Streptavidin Coated Fluorescent Magnetic Particles, Pink, 0.1% w/v, 8.84 µm, 2 mL).



Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Streptavidin, Fluorescent Yellow, High iron	0.2-0.39	0.1	FSVM-02552-2H	2 mL
Streptavidin, Fluorescent Nile Red	0.2-0.39	0.1	FSVM-02556-2	2 mL
Streptavidin, Fluorescent Yellow, High Iron	0.4-0.69	0.1	FSVM-0552-2H	2 mL
Streptavidin, Fluorescent Pink	1.0-1.4	0.1	FSVM-1058-2	2 mL
Streptavidin, Fluorescent UV/Light Yellow	2.0-2.9	0.1	FSVM-2042-2	2 mL
Streptavidin, Fluorescent Pink	2.0-2.9	0.1	FSVM-2058-2	2 mL
Streptavidin, Fluorescent Yellow	8.0-9.9	0.1	FSVM-8052-2	2 mL
Streptavidin, Fluorescent Pink	8.0-9.9	0.1	FSVM-8058-2	2 mL

- Reference Page 67 for an example streptavidin coated fluorescent magnetic particles used during a magnetic separation
- Reference Page 68 for a list of the magnetic separators that can be used with coated fluorescent magnetic separators.

SPHERO™ Protein A Coated Magnetic Fluorescent Particles

- Reference Page 74 for the binding strength of Protein A to immunoglobulins from different species.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Protein A, Fluorescent Yellow	90-105	0.1	FPAM-100052-4	4 mL
Protein A, Fluorescent Yellow	180-210	0.1	FPAM-200052-4	4 mL

SPHERO™ Coated Ferromagnetic Particles

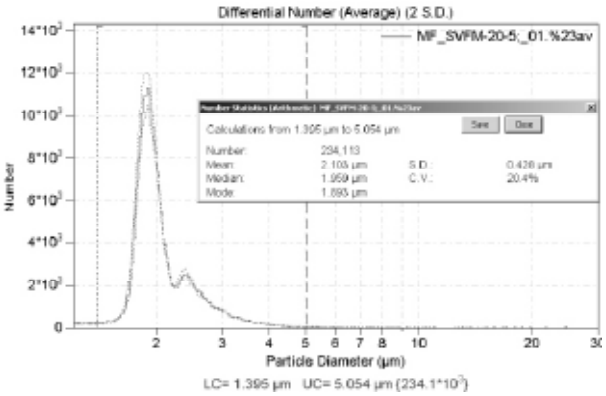
SPHERO™ Streptavidin Coated Ferromagnetic Particles

- See Page 70 for uses, benefits, and the mechanism of streptavidin coated particles
- Used for their ability to be easily manipulated in a magnetic field* .

*Anker, J. N., C. J. Behrend, et al. (2005). "Magnetically-modulated optical nanoprobes (MagMOONs) and systems." Journal of Magnetism and Magnetic Materials 293(1): 655-662

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Streptavidin	2.0-2.9	1.0	SVFM-20-5	5 mL
Streptavidin	4.0-4.9	1.0	SVFM-40-5	5 mL
Streptavidin	8.0-8.9	1.0	SVFM-80-4	4 mL
Streptavidin	10.0-13.9	1.0	SVFM-100-4	4 mL

Figure 115 Size distribution analysis of SPHERO™ Cat. No. SVFM-20-5, Streptavidin Ferromagnetic Particles from the Beckman Coulter Z3 Multisizer™ 3.



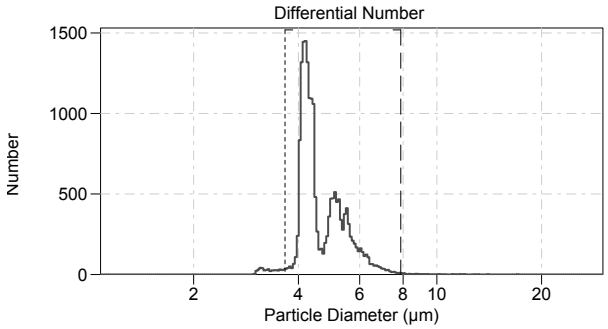
SPHERO™ Goat anti-Mouse IgG Coated Ferromagnetic Particles

- Provides a means to measure forces applied to a specimen through specific receptors proteins
- Aids in the development of magnetic systems designed to apply forces or force patterns
- Coated fluorescent ferromagnetic particles allows for force measurements using fluorescent microscopy
- Contains two orders of magnitude higher magnetic moments than paramagnetic particles and can be incubated with biological cells during phagocytosis assays*.

*Mitrelias, T., J. Palfreyman, et al. (2007). "Biological cell detection using ferromagnetic microbeads." Journal of Magnetism and Magnetic Materials 310(2, Part 3): 2862-2864.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Goat anti-Mouse IgG (Fc)	2.0-2.9	1.0	FMFc-25-5	5 mL
Goat anti-Mouse IgG (Fc)	4.0-4.5	1.0	FMFc-40-5	5 mL
Goat anti-Mouse IgG (H&L) , Cross adsorbed	2.0-2.9	1.0	FMXA-25-5	5 mL
Goat anti-Mouse IgG (H&L) , Cross adsorbed	4.0-4.9	1.0	FMXA-40-5	5 mL
Goat anti-Mouse IgG (Fc), Fluorescent, Pink	2.0-2.4	0.1	FMMFc-2058-5	5 mL

Figure 116 Size distribution analysis of SPHERO™ Cat. No. FMFc-40-5, Gt anti-Ms IgG (H&L) Ferromagnetic Particles, Lot AC01, 4.5 µm, 1.0 w/v, 5mL from the Beckman Coulter Z3 Multisizer™ 3.



Technical Information

The following **SpheroTECHNICAL NOTES** (STN) are available online and upon request:

STN-1: Particles Coating Procedures

STN-2: Determination of Antibody binding to particles

STN-3: Binding Capacity of Avidin Magnetic Particles

STN-4: Binding Capacity of Gt-anti-Ms-IgG Magnetic Particles

STN-5: Binding Capacity of Streptavidin Magnetic Particles

STN-6: Binding Capacity of Biotin Magnetic Particles

STN-7: Separation of Mononuclear Cells from Peripheral Blood using SPHERO™ Gt-anti-Ms-IgG Magnetic Particles

STN-8: Calibration and Performance Tracking of Flow Cytometer Using SPHERO™ Calibration Particles

STN-9: Measuring MEF with Flow Cytometer Using SPHERO™ Rainbow Calibration Particles.

STN-10: Magnetic Particles Enzyme Immunoassay (MPEIA) using UltraMag Separator System (UMS-4000)

STN-11: Magnetic Particles Coated with Pepsin, Papain and Trypsin

STN-12: Protein A Coated Magnetic Particles

STN-14: Determining PMT Linearity in Flow Cytometers Using the SPHERO™ PMT Quality Control Excel Template

STN-15: Measuring Absolute Cell Count Using SPHERO™ ACCUCOUNT Fluorescent Particles

STN-16: Covalent Coupling of Proteins to Microbeads Using a Heterobifunctional Coupling Agent

STN-17: Determination of a Flow Cytomerter's Sensitivity Using Detection Efficiency (Q) and the Background Light Level (B)

STN-18: Introduction to an Easy-To-Use Technique For The Setting of Flow Cytometer Compensation Using COMPtrol Antibody Capture Beads as a Subsitute For Cells

CHARACTERISTICS OF SPHERO™ POLYSTYRENE PARTICLES

Density: 1.05 **Refractive Index:** 1.59
Composition: Linear polystyrene
Shape: Uniform microspheres
Porosity: Nonporous
Compatibility with organic solvent: Inert to alcohol and DMSO but soluble in DMF, acetone, acetonitrile, xylene, chloroform and methylene chloride, etc.
Functional groups: Located on the surface with alkyl linker arm.
Functional group contents:
0.8 µm Carboxyl particles: ~ 50 µeq/g solid
0.8 µm Amino particles: ~ 20 µeq/g solid

Calculation of area / functional group: 1µm = 10^4Å, 1nm = 10Å
Number of 0.8µm particles per g = 3.5x10^12 particles/g
Surface Area / Particle = $4pr^2$ or pd^2
= $(0.8\ \mu m)^2 \times 3.14$
= $(0.8 \times 10^4 Å)^2 \times 3.14$
= $(0.64 \times 10^4 Å)^2 \times 3.14$
= $2 \times 10^8 Å^2$
# of COOH at 50 µeq = $50x10^{-6} \times 6.023x10^{23} = 3x10^{19}$ COOH/g
# of COOH / particle = $3x10^{19}$ COOH/g / $3.5x10^{12}$ particles/g
= $8x10^6$ COOH/particle
Surface Area / group = $2x10^8 Å^2$ / $8x10^6$ COOH/particle = ~$25Å^2$

AVIDIN POLYSTYRENE PARTICLES (VP-08-10, Lot W01, 0.88 µm):
Covalently coated with egg white avidin
Avidin Contents : ~ 14 µg/mg solid; ~ 0.212 nmole/mg solid
Binding capacity to Biotin-FITC: ~ 0.37 nmole/mg solid
Number of AV/bead :
$0.37x10^9 \times 6.023x10^{23} / 3.5x10^9 = 6.4x10^4$
$6.4x10^4 / 2 = 3.4x10^4$ AV/bead
NOTE: Theoretically free AV has 4 binding sites, but coated AV has only 2 Biotin binding sites available., MW of AV = ~66000, MW of Biotin-FITC = ~573.64, $3.5x10^9$ particles/mg

ANTIBODY COATED POLYSTYRENE PARTICLES (0.8 µm):
Antibody contents: ~ 14 µg/mg solid; ~ 0.2 µg/cm^2, ~$1.5x10^4$ IgG/particle
Binding capacity to IgG-FITC: ~ 4 µg/mg

The number of binding sites can be calculated by the binding capacity listed on the Technical Data Sheet.
eq: MPFc-30-5, Lot X01 has a binding capacity of 1.6µg/mg of particles. The number of binding sites per particle can be calculated as follows:
$(1.6x10^{-6}/160500) \times 6.023x10^{23} = 5.96x10^{12}$ IgG-FITC/mg of particles
Number of 3.2µm/per mg = $55.5x10^6$ particles/mg
# of IgG-FITC/particle = $5.96x10^{12}$ / $55.5x10^6$ particles/mg =
= $1.07x10^5$ IgG-FITC/particle.

NOTE: (MW of IgG = ~160500). The binding capacity is determined by binding a known concentration of IgG-FITC to a known amount of particles.

VOLUME, NUMBER AND SURFACE AREA vs. DIAMETER OF PARTICLES

Volume of particles: V = $(\pi/6) \times D^3 \times 10^{-12}$ cm^3/particle
Number of particles: N = $(6W/\pi PD^3) \times 10^{12}$ particles
Surface area of particles: A = $(6W/PD) \times 10^4$ cm^2
Where : W = Weight of polymer in gram
P = Density of polymer (polystyrene = 1.05)
D = Diameter of particles in micrometer
Total surface area of 1 mL of 5% w/v (50 mg) particles: A = 2857/D cm^2
Total surface area of 20 µL of 0.25% w/v (50 µg) particles: A = 2.857/D cm^2
1 µm = 10^4Å : 1 cm = 10^8Å : 1 cm^2 = 10^{16}Å^2 : 1 cm = 10^4µm

For 1 mL of 1% w/v (10 mg) particles

DIAMETER(µm)	SURFACE AREA(cm^2)	NUMBER($\times 10^9$)	A/Particle (Å$^2 \times 10^8$)	COOH (µeq/g)	NH_2 (µeq/g)
0.05	11428	145513	0.00785	800	320
0.10	5714	18189	0.0314	400	160
0.20	2857	2273.6	0.1253	200	80
0.30	1905	673.6	0.283	133.33	53.33
0.40	1429	284.2	0.5024	100	40
0.50	1143	145.51	0.785	80	32
0.80	714	35.53	2.01	50	20
1.0	571	18.19	3.14	40	16
1.5	381	5.389	7.065	26.67	10.67
2.0	286	2.274	12.56	20	8
2.5	229	1.164	19.625	16	6.4
3.0	190	0.6737	28.26	13.33	5.33
3.5	163	0.4242	38.465	11.43	4.57
4.0	143	0.2842	50.24	10	4
4.5	127	0.1996	63.585	8.89	3.56
5.0	114	0.1455	78.5	8	3.2
5.5	104	0.1093	94.99	7.27	2.91
6.0	95.0	0.0842	113.04	6.67	2.67
6.2	92.1	0.0763	120.7	6.45	2.58
6.7	85.2	0.0605	140.95	5.97	2.39
7.0	81.5	0.053	153.86	5.71	2.29
7.7	74.2	0.0398	186.17	5.19	2.08
8.0	71.4	0.035	200.96	5	2
10.0	57.1	0.018	314	4	1.6
15.0	38.1	0.0054	706.5	2.67	1.07
20.0	28.55	0.00227	1256	2	0.8
30.0	19.03	0.00067	2826	1.33	0.53
40.0	14.28	0.000284	5024	1	0.4
50.0	11.42	0.000145	7850	0.8	0.32
75.0	7.61	0.0000431	17662.5	0.53	0.21
100.0	5.71	0.0000182	3140	0.4	0.16
125.0	4.57	0.0000093	49062.5	0.32	0.128
150.0	3.80	0.0000054	70650	0.27	0.11
200.0	2.86	0.0000023	125600	0.2	0.08
300.0	1.90	0.00000067	282600	0.13	0.053

SPHERO™ RECOMMENDED PARTICLE COATING PROCEDURES

Introduction

Currently, there are several methods of attaching biological ligands to polystyrene particles. These methods include adsorption to plain polystyrene particles, covalent attachment to surface functionalized particles, and attachment of the ligand of interest to particles that are pre-coated with a binding protein such as Streptavidin, Protein A or Protein G. Presented in this technical information are protocols such as adsorption, covalent coupling, and other methods used to attach ligands to polystyrene particles.

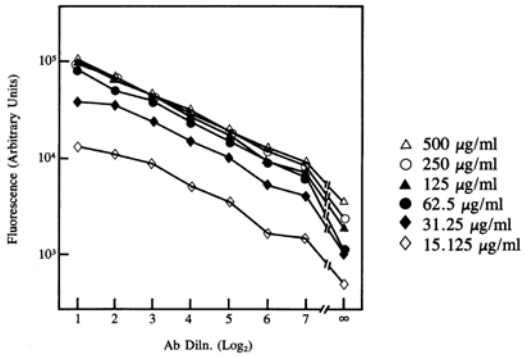
Procedures and Discussion

The following information explains generalized protocols for the attachment of ligands to polystyrene particles. These protocols are easily optimized to meet the requirements for specific applications. The following protocols are developed by Spherotech for the convenience of SPHERO™ microparticle users. **They are to be utilized only as initial conditions.** Spherotech encourages the optimization of the coating conditions by changing the buffer, pH or reagents concentration.

In general, polyclonal antibodies are coated to polystyrene particles by adsorption without using any coupling agents. The binding of polyclonal antibodies to polystyrene particles is strong. However, care should be taken not to over load the antibodies to the particles. If overloading occurs, leaching of the coated antibody will happen during storage. This is due to the weak interaction between antibody molecules compared to the interaction of antibody molecules to the surface of polystyrene particles. As shown in Fig. 1., the optimal antibody to particle ratio for passive adsorption is between 62.5 µg/mL to 125 µg/mL of 0.5% w/v (5.0 mg solid / mL) of 0.8 µm polystyrene particles[1].

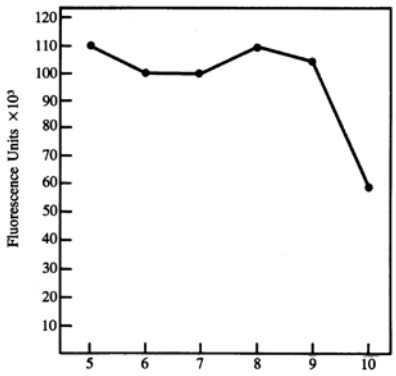
For 4.0~4.5 µm magnetic particles, the optimal antibody to particle ratio is around 250 µg/mL of 2.5% w/v magnetic particles. If different size particles are used, the antibody to particle ratio will need to be adjusted according to the surface area of the particles as compared to the 0.8 µm particles. When the same solid weight of particles are used the total surface area is inversely proportional to the size of the particles. For example, the same weight of 2.0 µm particles will have half as much of the surface area as that of 1.0 µm particles. Likewise, the same weight of 3.0 µm particles will have one third as much of the surface area as that of 1.0 µm particles. The buffer pH during passive adsorption of antibody to the particles can range from pH 5 to pH 9 as shown in Fig. 2., where pH 5.0 is sodium acetate, pH 6, 7 and 8 is phosphate and pH 9 and 10 is carbonate[1]. An acidic buffer of pH 5.0 such as MES, phosphate or acetate buffer is preferred for covalent coupling of proteins to carboxyl particles. Spherotech has used both acetate or phosphate buffer with EDC without encountering any problems.

Fig. 1. Effect of Coating Ligand Concentration on Quality of Assay Particles



Performance of polystyrene latex particles coated at pH 7.0 with various concentrations of human IgG.

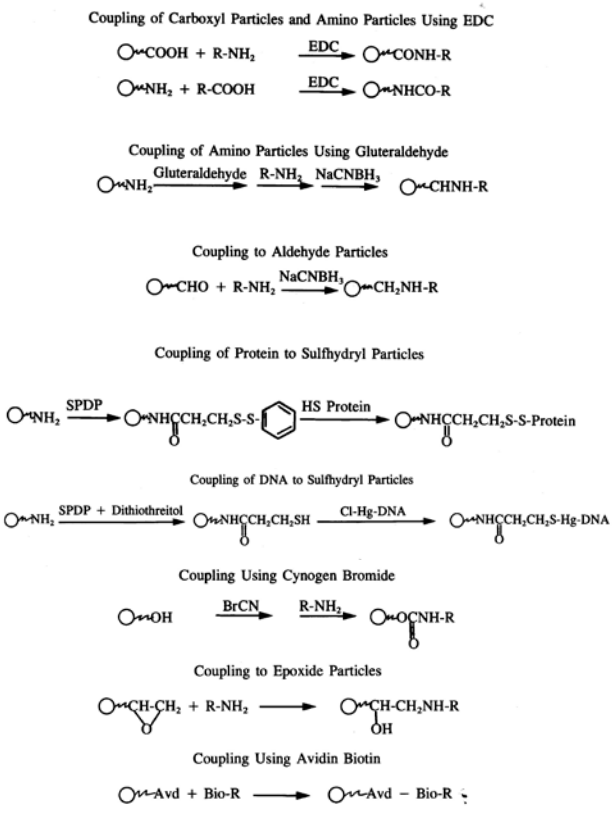
Fig. 2. Effect of Buffer pH on the \Quality of Particle Coating



Human IgG was incubated with plain polystyrene particles for 2 hours at ambient temperature. 0.1M buffers of varying pH (sodium acetate pH 5, sodium phosphate pH 6-8, sodium carbonate pH 9,10). Quality of coating was determined through PCFIA using labeled anti-human IgG.

Typically, centrifugation is used for removing unbound proteins after coating of polystyrene particles larger than 0.4 µm. On the contrary, gel filtration, dialysis or diafiltration is used for particles smaller than 0.4 µm. The magnetic particles are separated easily after the coating with a commercially available magnetic separator. The FlexiMag Separator or FlexiMag Separator, Jr., which are available from Spherotech, will provide a very convenient and cost effective way of cleaning magnetic particles after coating. A schematic presentation of covalent coupling of ligands to functionalized particles is shown in Fig. 3.

Fig. 3. Covalent Coupling of Ligands to Particles



General Procedures For Particle Coating

Passive adsorption:

1. Add the following to a 15 mL glass centrifuge tube:
   a. 1.8 mL of phosphate buffer, 0.1 M, pH 7.4
   b. 0.2 mL of 1 mg/mL protein solution
   c. 0.2 mL of 5% w/v 0.8 µm polystyrene particles
2. Vortex and incubate for at least one hour at ambient temperature
3. Centrifuge at 3000x g for 15 minutes
4. Remove the supernatant carefully
5. Add 4 mL of Isotonic Buffered Saline (IBS)
6. Mix well using a vortex mixer
7. Centrifuge at 3000x g for 15 minutes
8. Remove the supernatant carefully
9. Add 4 mL of IBS and mix well to obtain 0.25% w/v suspension.

Notes: 1. For 4.0~4.5 µm magnetic particles, use 250 µg of proteins per mL of 2.5% w/v magnetic particles and FlexiMag Separator orFlexiMag Separator, Jr. for the separation of particles in Steps 3 and 7

2. This procedure is used for the passive adsorption of immunoglobulins, antigens or other ligands to polystyrene or polystyrene magnetic particles.

Coating of Carboxyl Particles with Avidin Using EDC

Covalent Coupling (one step EDC coupling):

1. Add the following to a 15 mL glass centrifuge tube:
   a. 2 mL of sodium acetate buffer, 0.01 M, pH 5.0
   b. 2 mg of Avidin or Streptavidin
   c. 2 mL of 5% w/v 0.8 µm Carboxyl particles
   d. 20 mg of EDC
2. Vortex and incubate for two hours at ambient temperature on a rotary mixer or with occasional vortexing or shaking
3. Centrifuge at 3000x g for 15 minutes
4. Remove the supernatant carefully
5. Resuspend the pellet in 4 mL of Isotonic Buffered Saline
6. Repeat Steps 3 and 4 once and resuspend the pellet in 2 mL of IBS to obtain 2 mL of 0.5% w/v suspension.

Notes: 1. For 4.0-4.5 µm magnetic particles, use 0.5 mg of Avidin per 2 mL of 2.5% w/v magnetic particles and 5 mg of EDC. Use the FlexiMag Separator or Flex-iMag Separator, Jr. for the particle separation in Step 3

2. This procedure is also for covalent coupling of other proteins such as monoclonal or polyclonal antibodies, antigens or other ligands. Acidic buffers such as phosphate, (0.1M) or MES (0.05M) can also be used.

Coating of Amino Particles with Ligands or Proteins Using EDC

Covalent Coupling (one step EDC coupling):

1. Add the following to a 12x75 mL glass centrifuge tube:
   a. 2 mL of MES buffer, 0.05 M, pH 5.0
   b. 2 mg of ligands or proteins
   c. 2 mL of 5% w/v 0.8 µm Amino particles
   d. 20 mg of EDC
2. Vortex and incubate for two hours at ambient temperature on a rotary mixer or with occasional vortexing or shaking
3. Centrifuge at 3000x g for 15 minutes
4. Remove the supernatant carefully
5. Resuspend the pellet in 4 mL of Isotonic Buffered Saline
6. Repeat Steps 3 and 4 and resuspend the pellet in 2 mL of IBS to obtain 2 mL of 5% w/v suspension.

Notes: 1. For 4.0~4.5 µm magnetic particles, use 0.5 mg of ligands or proteins per 2 mL of 2.5% w/v magnetic particles and 5 mg of EDC. Use the FlexiMag Separator or FlexiMag Separator, Jr. for the separation of particles in Step 3.

2. For 1.0-2.0 µm magnetic particles, use 1.0 mg of ligands or proteins per mL of 2.5% w/v magnetic particles and 10 mg of EDC.

3. EDC(1-ethyl-3(-3-dimethylaminopropyl) carboiimide hydrochloride), Sigma Chemical Cat. No.

Covalent Coupling (two step EDC coupling):

For two step EDC coupling, wash the particles with coupling buffer, centrifuge and remove ~80% of the supernatant. Add EDC to the pellet, mix, and incubate for 1 hour. Wash the particles with coupling buffer and resuspend with protein solution. Continue with Steps 2 to 6 of the Covalent Coupling (one step) procedure.

Coating of Avidin Particles with Biotinylated Proteins

Affinity Coupling:

1. Add the following to a 15 mL glass centrifuge tube:
   a. 2.0 mL of biotinylated protein (100 µg/mL protein) in sodium phosphate buffer (PBS), 0.1 M, pH 5.5
   b. 0.2 mL of Avidin coated polystyrene particles, 5% w/v
2. Vortex and incubate for at least one hour at ambient temperature
3. Centrifuge at 3000x g for 10 minutes
4. Remove the supernatant carefully
5. Resuspend the pellet in 4 mL of 0.1M PBS
6. Repeat Steps 3 and 4 once and resuspend the pellet in 4 mL of PBS to obtain 4 mL of 0.25% w/v suspension.

Notes: 1.This procedure is also used for coating biotin-polystyrene or magnetic particles with various avidin-protein conjugates or other avidin-ligand conjugates.

Coating of Protein to Hydroxyl Particles

Covalent Coupling using Cyanogen Bromide:

1. Add 2 mL of 1.25% w/v 0.8 µm hydroxyl polystyrene particles to a centrifuge tube
2. Adjust the pH to 10.5 with 1N NaOH
3. Add 10 mg of CNBr in a fume hood
4. Readjust the pH to 10.5 with 1N NaOH
5. Incubate for 15 minutes
6. Add 2 mL of cold borate buffer (0.1M, pH 8.5)
7. Cool to 4°C and add 1 mL of protein at a concentration of 1 mg/mL
8. Incubate at 4°C for at least four hours
9. Add 5 mL of glycine buffer (0.1M, pH 8.5)
10. Centrifuge for 30 minutes at 2000x G
11. Remove the supernatant and resuspend the pellet in 10 mL of 0.1M phosphate buffer, pH 7.2
12. Repeat Steps 10 and 11 twice to give 10 mL of particles at 0.25% w/v.

Coating of Dimethylamino Particles with DNA

Ionic Interaction Coupling:

1. Add the following to a 15 mL glass centrifuge tube:
   a. 100 µL of 0.25% w/v 0.8 µm dimethylamino particles in carbonate buffer, 0.1 M, pH 9.0
   b. 5.0 µL of DNA (200 ng/mL) in 0.1M carbonate buffer, pH 9.0
2. Vortex and incubate for three hours at ambient temperature
3. Centrifuge at 3000x g for 15 minutes
4. Remove the supernatant and resuspend the pellet in 150.0 µL of 0.1M carbonate buffer, pH 9.0
5. Centrifuge at 3000x g for 15 minutes
6. Remove the supernatant and resuspend the pellet in 100.0 µL of 0.1M Tris buffer, pH 7.5. Final particle concentration is 0.25% w/v. Store refrigerated.

Coating of Ligands to Modified Amino Proteins

Covalent Coupling Using SPDP[2]:

2-Pyridyldisulfide particles:

1. Add the following to a 15 mL glass centrifuge tube:
   a. 5.0 mL of sodium phosphate buffer 0.1 M, pH 7.0
   b. 5.0 mL of amino polystyrene particles, 0.8 µm, 5% w/v
   c. 0.5 mL of DMSO containing 12.5 mg of SPDP [3-(2-pyridyldithio) propionic acid N-hydroxy succinimide ester)]
2. Incubate for at least one hour at ambient temperature on a rotary mixer
3. Centrifuge at 3000x g for 30 minutes
4. Remove the supernatant carefully
5. Resuspend the pellet in 5 mL of 0.1M PBS, pH 7.0
6. Centrifuge at 3000x g for 30 minutes
7. Repeat Steps 3 and 4 and resuspend the pellet in 5 mL of deionized water. Store at 4°C.

Thio Ester Particles:

1. Repeat Steps 1-5 from the **Covalent Coupling Using SPDP[2]** procedure, or use the suspension obtained in Step 6 and centrifuge at 3000x g for 30 minutes
2. Remove the supernatant carefully
3. Resuspend the pellet in 5 mL of 0.1M acetate buffer, pH 5.0
4. Add 40 mg of DTT (1,4-dithiothreitol)
5. Incubate for 30 minutes at ambient temperature on a rotary mixer
6. Centrifuge at 3000x g for 30 minutes
7. Aspirate and save the supernatant. If cloudy, filter through a 0.22 µm Acrodisk. Save the filtrate for optical density (OD) measurement at 343 nm. Multiply the absorbance by 8.08×10^3 to obtain the molar concentration of the thio ester on the particles
8. Resuspend the pellet in deionized water
9. Centrifuge at 3000x g for 30 minutes
10. Remove the supernatant and resuspend the particles in 5 mL of deionized water to give a 5% w/v suspension of thio particles. Use the thio particles as soon as possible for coupling to thiolated ligands. The thio groups can be oxidized to disulfide groups upon prolonged storage.

Modification of Ligands with SPDP and Coupling to Thio Particles:

1. Add a solution containing 1 mg of SPDP in 0.5 mL of methanol to a solution containing 4 mg of ligand in 2 mL of phosphate buffer (PBS), 0.1M, pH 7.0
2. Incubate for at least one hour at ambient temperature on a rotary mixer
3. Dialyze the mixture using a dialysis tubing of appropriate molecular weight cut off against three changes of PBS in 24 hours
4. Add the resulting thio ligand to 2 mL of 5% w/v thio particles
5. Incubate overnight at ambient temperature on a rotary mixer
6. Centrifuge at 3000x g for 30 minutes
7. Remove the supernatant and resuspend the particles in 10 mL of PB
8. Repeat Steps 6 or 7 and centrifuge at 3000x g for 30 minutes
9. Remove the supernatant and resuspend the particles in 40 mL of PBS to give a 0.25% w/v suspension.

Coating of Carboxyl Particles with Amino Modified Oligonucleotides

1. Add 2.5x10^6 carboxyl polystyrene particles to 62 µL of 0.1M MES (2-[N-morpholino]ethanesulfonic acid)
2. Add 5 nmoles of amino modified oligonucleotide in 25 µL of 0.1M MES
3. Add 0.3 mg of EDC(1-ethyl-3(-3-dimethylaminopropyl) carboiimide hydrochloride).
4. Vortex and incubate for 20 minutes at ambient temperature
5. After 20 minutes add 0.3 mg of EDC
6. During incubation occasionally mix to avoid sphere clumping
7. After another 20 minutes add 0.3mg of EDC
8. During incubation occasionally mix to avoid sphere clumping
9. Incubate for another 80 minutes on a rotary mixer
10. Centrifuge and remove the supernatant carefully
11. Resuspend the pellet in 1 mL of 0.1M PBS containing 0.02% Tween-20
12. Repeat Step 10 and resuspend the pellet in 150 µL of 10 mM Tris [hydroxymethyl]aminomethane hydrochloride / 1mM EDTA (ethylenediamine-tetraacetic acid) pH 8.0 (TE).
11. Centrifuge and remove the supernatant carefully
12. Resuspend the pellet in 200 µL of TE or IBS. Store at 4°C.

Periodate Oxidation of Polysaccharide and Coupling to Amino Particles

1. Add a solution containing 1 mg of sodium m-periodate (Sigma, cat. No. S-1878) in 1 mL of di water dropwise with stirring to a solution containing 10 mg of polysaccharide in 2 ml of di water
2. Stir the mixture at room temperature for 30 minutes and add 10 µL of 1 M ethylene glycol to the mixture. After five minutes, add the mixture to the packed 0.8 µm amino polystyrene particles obtained from 5 mL of 5% w/v suspension by centrifugation at 4000x g for 10 minutes
3. Adjust the pH of the mixture to 9.0 to 9.5 with 10% K_2CO_3 and stir the mixture at room temperature for at least 45 minutes
4. Add 6 mg of sodium cyanoborohydride (Sigma S-8628) to the mixture and stir the mixture at room temperature overnight
5. Wash the particles twice with 5 mL of deionized water and resuspend the particles in 5 mL of 0.1 M PBS containing 100 mg of BSA
6. Stir the mixture at room temperature for two hours and wash the particles twice as before
7. Resuspend the particles in 5 mL of 0.1M PBS to give 5 mL of 5% w/v suspension.

Conclusion

Proteins and ligands adsorption onto polystyrene readily and permanently. Coating the surface of polystyrene particles with proteins is successful most of the time using adsorption techniques. Adsorption is adequate for the coating of most polyclonal IgG for assay systems. Other methods of coating particles can be considered if simple adsorption is inadequate. A wide array of particle coating mechanisms with different surface chemistries of particles are now available. For instance, monoclonal antibodies with low isoelectric points will require covalent coupling. Figure 3 will show the mechanism for other methods of coating particles. In this technical note, Spherotech has recommended different initial procedures for the production of tests and assays that provide good sensitivity and stability using polystyrene particles.

Important Notes:

1. Since the quality of the coated particles depends on the quality of reagents and on the coating procedures, high quality reagents should be used while optimizing the coating conditions. As a result of Spherotech's lack of control over the reagents and coating condition, Spherotech can not guarantee the quality or performance of the coated particles even if the provided procedures are followed.

2. Isotonic Buffered Saline (IBS) is prepared using the following formula:

NaCl	8.0g
KCl	0.28g
$NaHPO_4$	0.275g
Na_2HPO_4	2.021g
Sodium Azide	0.2g
Deionized Water	1000mL

References

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