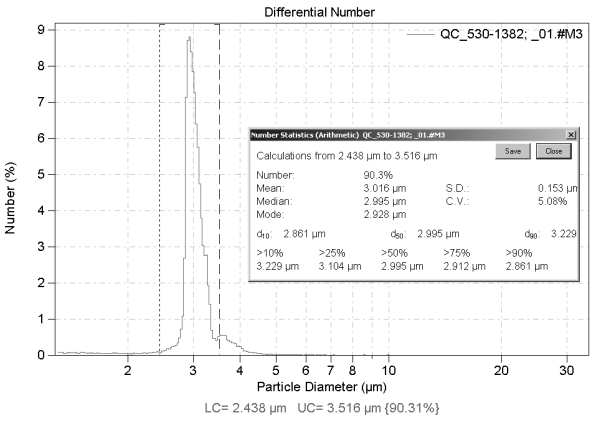
SPHERO™ Coated Particles

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

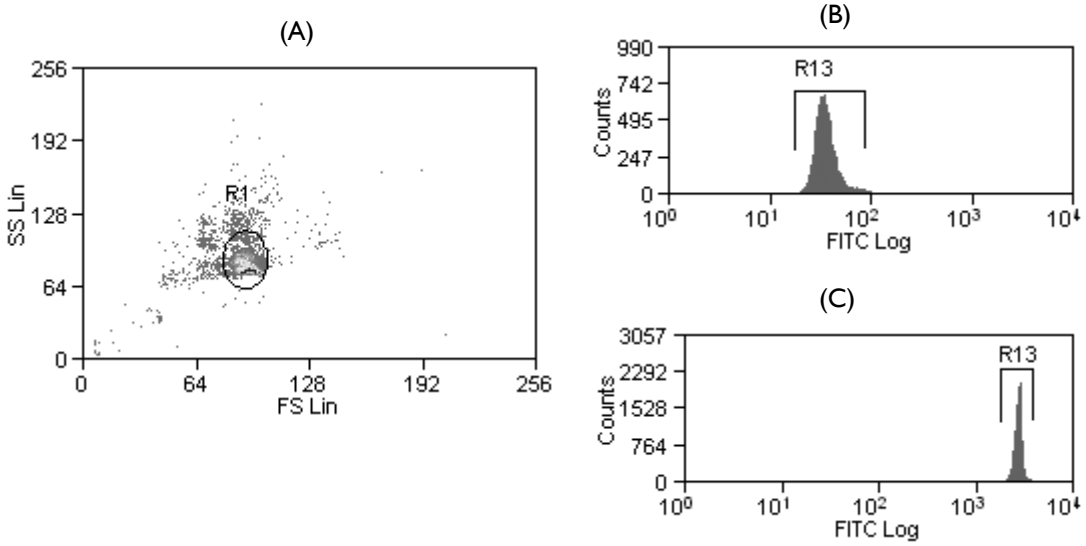


The SPHERO™ Coated Particles are prepared either by passive adsorption or covalent coupling, depending upon the intended applications. For example, the 4.0-4.5 µm Goat anti-Mouse IgG, Goat anti-Rabbit IgG and Goat anti-Human IgG coated magnetic particles intended for cell separation are prepared by covalent 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 83 or *www.Spherotech.com/tech* SpheroTechnical Note 1 for more detailed technical information regarding coating procedures.

Figure 91 (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	0.3-0.39	1.0	SVBP-03-10	10 mL
Streptavidin, Cross-linked	0.7-0.9	1.0	SVPX-08-10	10 mL

Figure 92 Size distribution analysis of SPHERO™ Cat. No. SVP-05-10 (Streptavidin Polystyrene Particles, 1% w/v, 0.54 µm, 10 mL, Lot AC01)

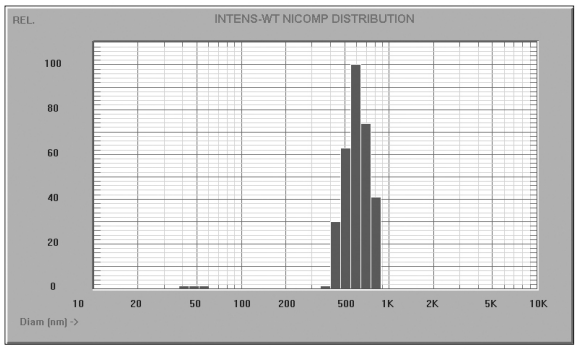
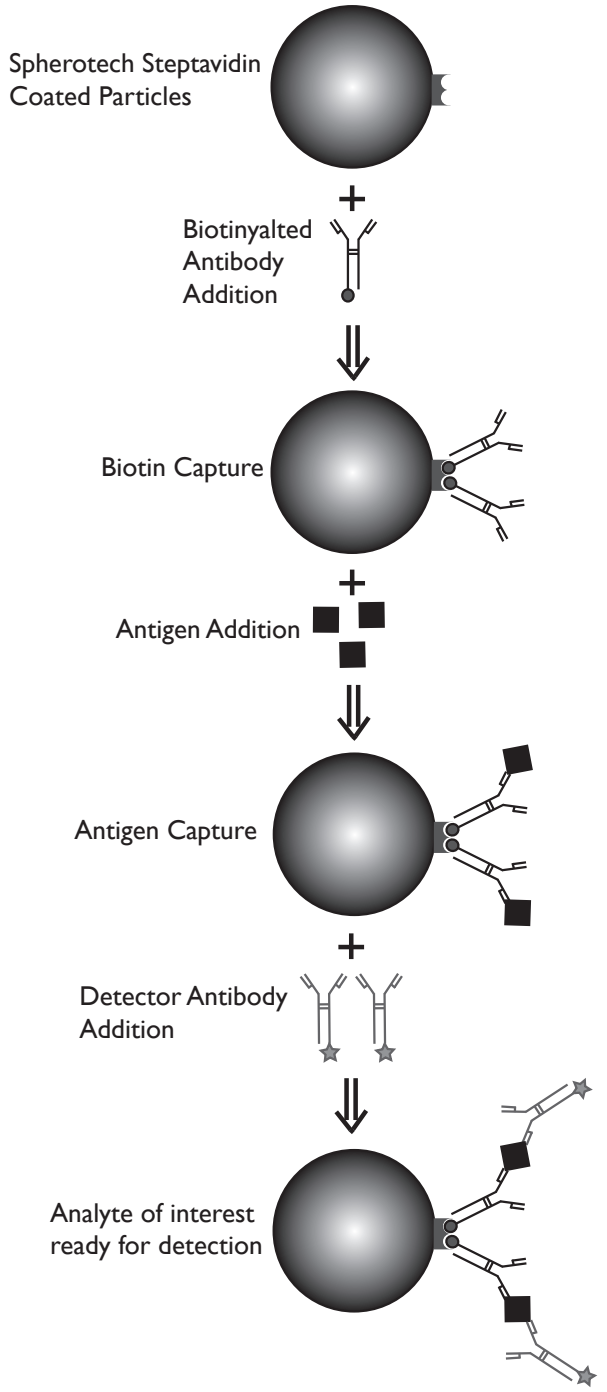


Figure 93 Example of streptavidin coated particles used in an sandwich immunoassay with a biotinylated antibody, antigen and labelled antibody.



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 94 (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 AA01.

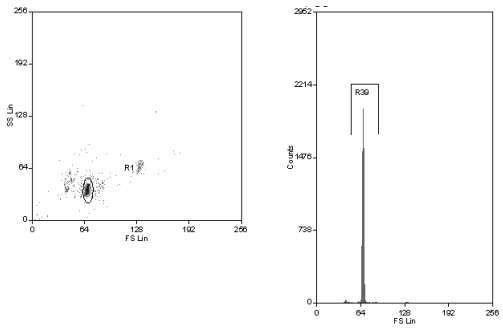
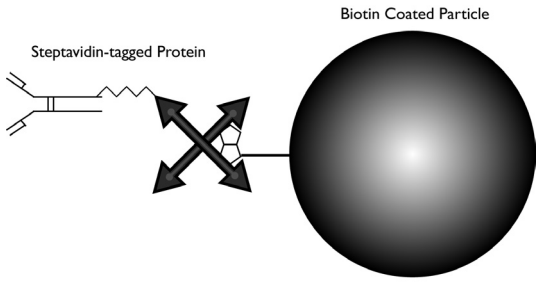


Figure 95 Example of biotin coated particles bound to streptavidin-tagged protein.

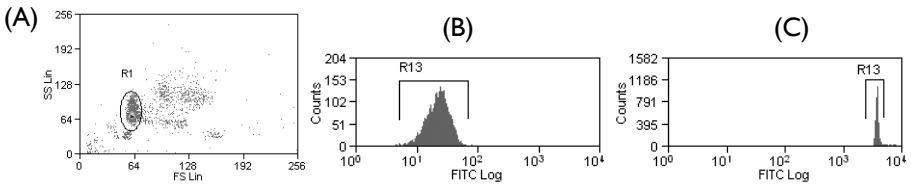


SPHERO™ Goat anti-Mouse IgG Coated Particles

- 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 96 (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 ~5.13 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	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