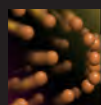
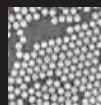


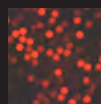
Thermo Scientific Particle Technology Product Catalog and Technical Reference Guide



Magnetic Particles



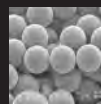
Size Standards and Count Control Particles



Flow Cytometry Particles



Dyed and Fluorescent Particles



**Clinical Diagnostic and Specialty Application
Particles**

Thermo Scientific Product Categories

Magnetic Particles

- Sera-Mag SpeedBeads
- Sera-Mag Magnetic Particles

Size Standards and Count Control Particles

- Duke Standards
- Certified Count Particles

Flow Cytometry Particles

- Cyto-Cal Calibration and Set-up Particles
- Cyto-Plex Surface-Modified Particles for Multiplex Assays

Dyed and Fluorescent Particles

- Color-Rich Dyed
- ChromoSphere Dyed
- Fluoro-Max Fluorescent

Clinical Diagnostic and Specialized Application Particles

- Opti-Bind
- Opti-Link
- Power-Bind Streptavidin-Coated
- Polymer Particle Suspensions
- Co-Polymer Particle Suspensions
- Smoke Detector Particles

Thermo Scientific Particle Technology

The source of particles for your diagnostic, calibration and molecular biology needs

With a depth and breadth of innovative particle technology design and manufacturing experience that spans over 35 years, we are your proven, reliable source for world class particle technology solutions including:

- Magnetic particles
- Size standards (NIST Traceable)
- Count control particles
- Flow cytometry particles
- Dyed and fluorescent particles
- Plain particles
- Non-polymer particles
- Cleanroom particles

Typically, engineers, physicists, chemists and scientists at several of the world's leading universities, research institutions and clinical diagnostic labs use these Thermo Scientific particles for or as:

- Molecular biology applications
- Reference standards for size measurement
- Nucleic acid isolation and cell separation
- Unique markers for biomolecules
- Flow tracing devices
- Reactive surfaces for diagnostic reagents
- Various other applications

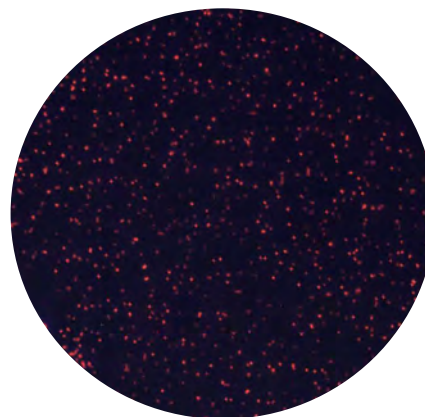
All Thermo Scientific particles are manufactured in our proprietary ISO 13485 certified facilities, resulting in products that provide:

- Superior precision and accuracy
- Low non-specific binding
- High binding capacity
- Excellent reproducibility
- Long-term stability

By manufacturing our own particles, we can provide customers with comprehensive data about the characteristics and functionality of the particles they purchase.

Customers can also count on our responsive, technical support backed by years of clinical applications training and research.

To learn more, visit www.thermoscientific.com/particletechnology or contact our technical support team at 1-800-232-3342 (USA), 1-510-979-5000 (International), or info.microparticles@thermofisher.com

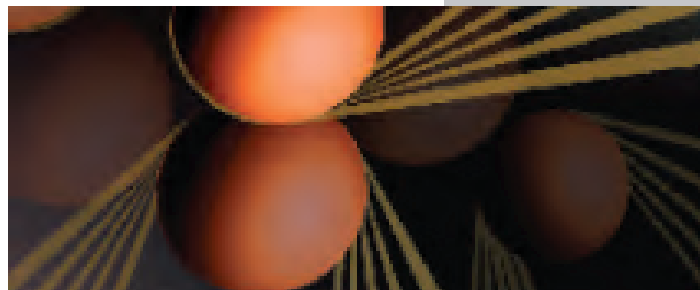


Did you know?

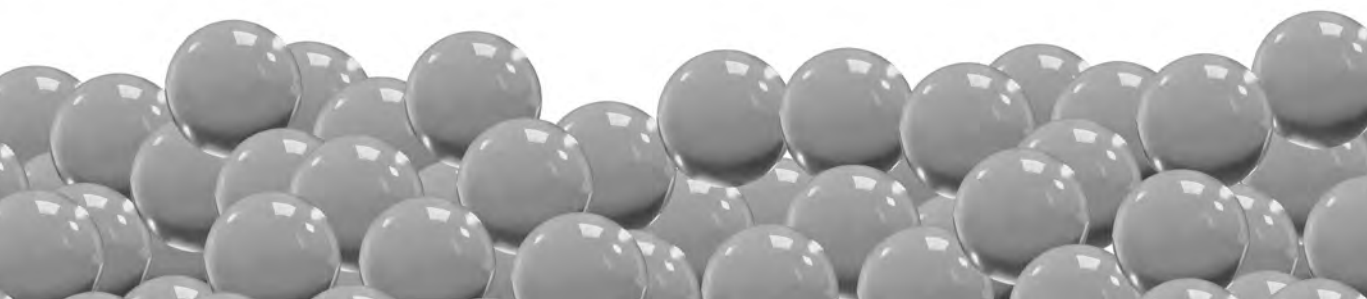
Approximately 85% of the U.S. blood supply is tested by assays that utilize Thermo Scientific particles.



Table of Contents

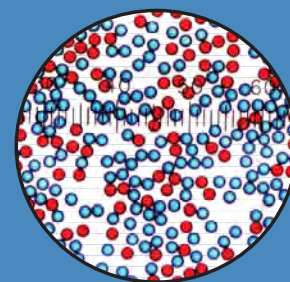


Particle Application Chart.....	2-3
Sera-Mag Magnetic Particles.....	4
Sera-Mag Magnetic SpeedBeads Particles.....	5-6
Sera-Mag Magnetic Particles.....	7-9
Technical Supplement: Particle Reagent Optimization.....	10-15
Size Standards and Count Control Particles.....	16
NIST Traceable Size Standards.....	17-25
Count Controls.....	26-29
Technical Supplement: Working with Particles.....	30
Flow Cytometry Particles.....	31
Calibration and Set-up Particles (Cyto-Cal).....	32-34
Multiplex Assay Particles (Cyto-Plex).....	35-36
Technical Supplement: Differences in Particles for Flow Cytometry.....	37
Dyed and Fluorescent Particles.....	38
Dyed Particles.....	39-40
Fluorescent Particles.....	41-47
Technical Supplement: Sonication and Mixing.....	48-50
Clinical Diagnostic and Specialty Application Particles.....	51
Particles for Clinical Diagnostics.....	52-55
Particles for Specialty Applications.....	56-58
Technical Supplement: Selecting Particle Surface Properties.....	59
Meeting Your Particle Technology Needs.....	60-61
Ordering Information, Sample Evaluation Packets, Returns, Payment, MSDS, Quality Commitment, Technical Support, Customer Service	



Particle Applications	MG SB CM	MG SB SA	MG SB NA	MG-CM	MG-SA	MG-OL	NIST Size Std.	Count Controls	Cyto-Cal	Cyto- Plex	Fluoro- SF GR, RD, BL	Fluoro- CM-Eu	Fluoro- SA-Eu
Air sampling smoke detectors													
Affinity purification	●	●	●	●	●								
Analytical, sedimentation, separation							●						
Biotinylated PCR isolation		●	●		●								
Biosensors	●	●	●	●	●	●							
cDNA libraries						●							
Cell isolation and purification	●	●	●	●	●	●							
Cell labeling										●		●	●
Cell sorting									●	●			
Cell surface markers												●	●
Chemiluminescent assays	●	●	●	●	●								
Contamination control/ flow tracing											●		
Cycle sequencing cleanup	●			●									
Dispersion studies							●	●			●	●	
Filter checking and testing/ challenge							●				●		
Filtration systems							●				●		
Fluid mechanics							●				●		
Fluorescent-based assays													●
Flow cell focusing									●				
Gene expression analysis/genotyping	●	●	●	●	●	●							
High sensitivity assays	●	●	●	●	●							●	●
Immunoassays	●	●	●	●	●							●	●
Instrument calibration/validation							●	●	●				
Instrument precision monitoring							●	●	●				
Laser particle counter calibration							●	●	●				
Lateral flow assays												●	●
Light scattering							●	●					
Membrane-based assays												●	●
Molecular diagnostics	●	●	●	●	●	●							
Multiplex bead assays										●			
Nephelometric assays													
Northern analysis		●	●		●	●							
Nucleic acid sample preparation	●	●	●	●	●	●							
Optical alignment							●	●	●				
Particle counting measurement							●	●	●				
Particle sizing							●	●					
Phagocytosis studies											●		
Purify PCR product	●	●	●	●	●								
Pore size determination							●				●		
Rapid assays												●	●
RT-PCR		●	●		●	●							
Slide agglutination assays												●	●
Suspension array analysis										●			
Time-resolved fluorescent assays												●	
Traceability of analytical methods							●	●					
Turbidimetric assays													

Color-Rich Blue	Color-Rich Red	Color Rich Black	Chromo Sphere RD,BLK	Opti-Bind SF	Opti-Link CM	Modified Undyed SF	Power-Bind SA	Smoke-Check	Particle Applications
								●	Air sampling smoke detectors
							●		Affinity purification
									Analytical, sedimentation, separation
									Biotinylated PCR isolation
					●	●			Biosensors
									cDNA libraries
									Cell isolation and purification
									Cell labeling
									Cell sorting
									Cell surface markers
					●	●	●		Chemiluminescent assays
●	●	●						●	Contamination control/ flow tracing
									Cycle sequencing cleanup
●	●	●		●	●				Dispersion studies
●	●	●		●	●	●		●	Filter checking and testing/ challenge
●	●	●		●	●	●			Filtration systems
●	●	●		●	●				Fluid mechanics
									Fluorescent-based assays
									Flow cell focusing
									Gene expression analysis/genotyping
●	●	●		●	●	●	●		High sensitivity assays
●	●	●		●	●		●		Immunoassays
									Instrument calibration/validation
									Instrument precision monitoring
									Laser particle counter calibration
●	●	●	●				●		Lateral flow assays
									Light scattering
●	●	●	●	●	●	●	●		Membrane-based assays
									Molecular diagnostics
									Multiplex bead assays
				●	●	●	●		Nephelometric assays
									Northern analysis
							●		Nucleic acid sample preparation
									Optical alignment
									Particle counting measurement
									Particle sizing
●	●	●		●		●			Phagocytosis studies
									Purify PCR product
				●		●			Pore size determination
●	●	●	●	●	●	●	●		Rapid assays
									RT-PCR
●	●	●	●	●	●	●			Slide agglutination assays
									Suspension array analysis
									Time-resolved fluorescent assays
									Traceability of analytical methods
				●	●	●	●		Turbidimetric assays



Particle Application Chart

Abbreviation Key

- SF: Sulfate
- CM: Carboxylate-modified
- SA: Streptavidin coated
- OL: Oligo(dT) coated
- NA: NeutrAvidin coated
- RD: Red
- BL: Blue
- BLK: Black
- GR: Green
- Eu: Europium Chelate
- MG: Magnetic
- SB: SpeedBead
- Size Std.: Size Standards
- Fluoro: Fluoro-Max

Thermo Scientific Sera-Mag Magnetic Particles

Sera-Mag Magnetic SpeedBeads Particles

- Sera-Mag Magnetic SpeedBeads Protein A/G
- Sera-Mag Magnetic SpeedBeads Carboxylate-Modified
- Sera-Mag Magnetic SpeedBeads NeutrAvidin
- Sera-Mag Magnetic SpeedBeads Streptavidin
- Sera-Mag Magnetic SpeedBeads Streptavidin-Blocked
- Sera-Mag Magnetic SpeedBeads Amine-Blocked

Sera-Mag Magnetic Particles

- Sera-Mag Magnetic Carboxylate-Modified Particles
- Sera-Mag Magnetic Oligo(dT) Particles
- Sera-Mag Magnetic Streptavidin Particles

Thermo Scientific Sera-Mag Magnetic SpeedBeads Particles

Sera-Mag Magnetic SpeedBeads particles respond twice as fast in a magnetic field as our original Sera-Mag Magnetic Particles, and are available in carboxylate-modified, neutravidin- and streptavidin-coated versions, and also as a protein A/G particle. They are especially useful in clinical immunoassays where speed of magnetic response is important, and for isolation from viscous solutions in molecular biology applications.

Additional benefits include:

- Sensitivity
- Physical integrity
- Colloidal stability
- Reproducibility
- High binding capacity
- Slow settling rate in the absence of a magnetic field
- Not affected by conditions such as sonication, drying, freezing and pH extremes
- Effectiveness in clinical and molecular applications
- Cost effective and long shelf life

SpeedBeads Protein A/G

- Isolates IgA and IgG proteins in a single step instead of running two separate processes
- Presence of protein A/G binding sites accommodates broadest range of antibody species and sub-classes
- Lower non-specific binding for cleaner, faster purification

SpeedBeads Carboxylate-Modified

- Base magnetic SpeedBeads particle has two layers of magnetite for twice the speed
- Provides fast magnetic response time for clinical diagnostic assays
- Excellent for a variety of nucleic acid isolation applications

SpeedBeads Streptavidin

- Ideal for demanding applications that require high binding capacity
- Provides much quicker isolation from viscous cell lysates
- Universal base particle for streptavidin-biotin isolation systems

SpeedBeads NeutrAvidin

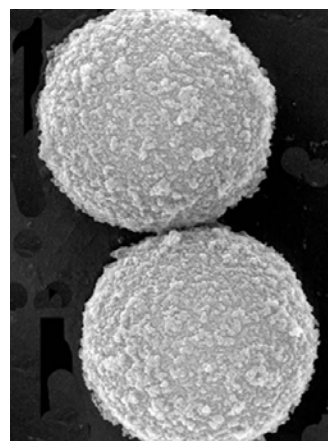
- Broad utility for a variety of high binding capacity needs
- Much quicker isolation from viscous cell lysates
- Potential lower non-specific binding characteristics

SpeedBeads Streptavidin- and Amine-Blocked

- Non-surfactant, non-protein blocked surface
- Significantly reduces undesired adsorption of proteins from a sample matrix

Differences in Encapsulated Levels for Sera-Mag Carboxylate-modified Particles

The 6515 and 4415 Sera-Mag SpeedBeads carboxylate-modified particles have surfaces that are more hydrophobic while the 4515 and 2415 have surfaces that are more hydrophilic. Some performance feedback has indicated that the 6515 and 4415 particles work better with nucleic acid isolation. To decide which is best for your specific application, it is suggested to purchase both as samples and perform an experiment to verify if there is a difference for your application.



Sera-Mag Magnetic SpeedBeads particles respond twice as fast in a magnetic field as our original Sera-Mag Magnetic particles, and are ideal for clinical and molecular diagnostics.

More SpeedBeads on next page...

Thermo Scientific Sera-Mag Magnetic SpeedBeads Particles

Sera-Mag SpeedBeads Protein A/G	Nominal Diameter	Bottle Size	Binding Capacity	Type/ Parking Area	Catalog Number
<i>Packaged in 1 mL, 5 mL, and 100 mL</i>	~1 µm	1 mL	High	55-85 µg IgG	1715-2104-011150
<i>1% solids, 10 mg/mL</i>	~1 µm	5 mL	High	55-85 µg IgG	1715-2104-010150
	~1 µm	100 mL	High	55-85 µg IgG	1715-2104-010350

Sera-Mag SpeedBeads Carboxylate-modified	Nominal Diameter	Bottle Size	Binding Capacity	Type/ Parking Area	Catalog Number
<i>Packaged in 15 mL, 100 mL, and 1000 mL</i>	~1 µm	15 mL	High	Carboxyl/PA5/0.05% Azide	4515-2105-050250
<i>5% solids, 50 mg/mL</i>	~1 µm	100 mL	High	Carboxyl/PA5/0.05% Azide	4515-2105-050350
	~1 µm	1000 mL	High	Carboxyl/PA5/0.05% Azide	4515-2105-050450
	~1 µm	15 mL	High	Carboxyl/PA5/0.05% Azide	6515-2105-050250
	~1 µm	100 mL	High	Carboxyl/PA5/0.05% Azide	6515-2105-050350
	~1 µm	1000 mL	High	Carboxyl/PA5/0.05% Azide	6515-2105-050450

Sera-Mag SpeedBeads NeutrAvidin-coated	Nominal Diameter	Bottle Size	Binding Capacity	Type/ Parking Area	Catalog Number
<i>Packaged in 1 mL, 5 mL, and 100 mL</i>	~1 µm	1 mL	High	Neutravidin surface/0.05% Azide	7815-2104-011150
<i>1% solids, 10 mg/mL</i>	~1 µm	5 mL	High	Neutravidin surface/0.05% Azide	7815-2104-010150
	~1 µm	100 mL	High	Neutravidin surface/0.05% Azide	7815-2104-010350

Sera-Mag SpeedBeads Streptavidin-coated	Nominal Diameter	Bottle Size	Binding Capacity	Type/ Parking Area	Catalog Number
<i>Packaged in 1 mL, 5 mL, and 100 mL</i>	~1 µm	1 mL	Medium	Streptavidin surface/0.05% Azide	6615-2104-011150
<i>1% solids, 10 mg/mL</i>	~1 µm	5 mL	Medium	Streptavidin surface/0.05% Azide	6615-2104-010150
	~1 µm	100 mL	Medium	Streptavidin surface/0.05% Azide	6615-2104-010350

Sera-Mag SpeedBeads Streptavidin-blocked	Nominal Diameter	Bottle Size	Binding Capacity	Type/ Parking Area	Catalog Number
<i>Packaged in 1 mL, 5 mL, and 100 mL</i>	~1 µm	1 mL	Medium	Streptavidin blocked/0.05% Azide	2115-2104-011150
<i>1% solids, 10 mg/mL</i>	~1 µm	5 mL	Medium	Streptavidin blocked/0.05% Azide	2115-2104-010150
	~1 µm	100 mL	Medium	Streptavidin blocked/0.05% Azide	2115-2104-010350

Sera-Mag SpeedBeads Amine-modified	Nominal Diameter	Bottle Size	Binding Capacity	Type/ Parking Area	Catalog Number
<i>Packaged in 1 mL, 5 mL, and 100 mL</i>	~1 µm	1 mL	Medium	Amine surface/0.05% Azide	1915-2104-011150
<i>1% solids, 10 mg/mL</i>	~1 µm	5 mL	Medium	Amine surface/0.05% Azide	1915-2104-010150
	~1 µm	100 mL	Medium	Amine surface/0.05% Azide	1915-2104-010350

Sample Packs

	Package Size	Sample Pack Includes:	Catalog Number
Sera-Mag SpeedBeads Carboxylate-modified	2 x 15 mL	4515-2105-050250 6515-2105-050250	S4565
Sera-Mag SpeedBeads Streptavidin and NeutrAvidin-coated	2 x 1 mL	6615-2104-011150 7815-2104-011150	S6678

Thermo Scientific Sera-Mag Magnetic Particles

World-Class Technology

The Sera-Mag family of super-paramagnetic particles are nominal 1 μm magnetic carboxylate-modified base particles (MG-CM) made by a core-shell process. They combine a fast magnetic response time with a large surface area and fast reaction kinetics.

Typically, these particles are used in various molecular biology, nucleic acid isolation, research and clinical diagnostic immunoassay applications.

Molecular Biology Applications

- Plasmid DNA isolation
- Genomic DNA isolation
- mRNA and PNA isolation
- Cell isolation
- Cycle sequence reaction cleanup
- Isolate biotinylated PCR product
- RT-PCR
- cDNA library construction
- Genotyping
- Subtractive hybridization
- Northern analysis
- Gene expression analysis

Clinical Diagnostics Applications

- Colorimetric assays
- Heterogeneous assays
- Chemiluminescent assays

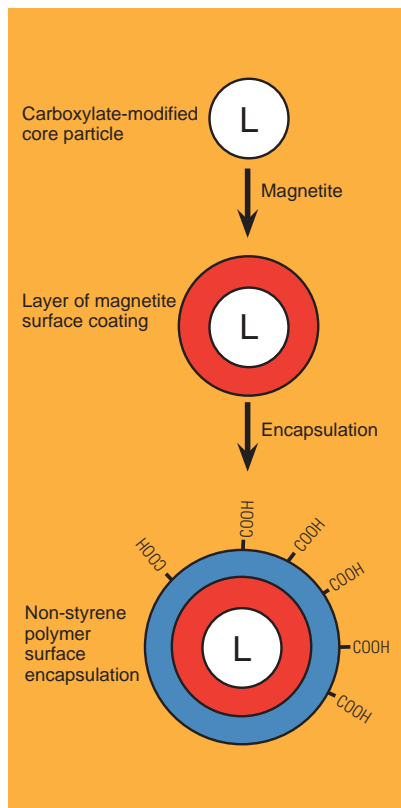
Unique Sera-Mag Properties

- Low, non-specific binding of serum proteins and other interfering substances
- Non-leaching, encapsulated magnetite
- Surfactant-free, no washing or pre-cleaning steps required
- High surface area per unit mass, high ligand binding capacity and slow settling rate in the absence of a magnetic field
- Tight size distribution provides simultaneous magnetic separation rate, efficient coating of biological reagents, and excellent lot-to-lot reproducibility
- GMP manufacturing in our ISO 13485 certified facilities
- Covalently bound ligands do not leach
- High yield
- Leads to pure sample preparations
- Rapid isolation in viscous solutions

Stability

- Stable in pH 1 to 13
- Stable in guanidinium thiocyanate
- Stable in DMF and DMSO
- Stable in sonication environments
- Stable in PCR temperature cycling
- 60 month shelf-life stability

Sera-Mag Magnetic Core-Shell Design



More on next page...

Thermo Scientific Sera-Mag Magnetic Particles

Sera-Mag Magnetic Streptavidin Particles

Sera-Mag magnetic streptavidin (MG-SA) particles contain covalently bound streptavidin and are available with low (2500 to 3500 pmol/mg), medium (3500 to 4500 pmol/mg) or high (4500 to 5500 pmol/mg) biotin binding capacities. These are measured in picomoles (pmol) of biotin-fluorescein bound per milligram of particle. The multiple levels let you choose the biotin-binding capacity needed to optimize your application. MG-SA can be used as a universal base particle for coating biotinylated proteins, oligos or other ligands to the particle surface. Shelf life stability is 60 months.

Sera-Mag Magnetic Carboxylate Particles

Sera-Mag magnetic carboxylate-modified particles feature low non-specific binding of serum proteins, high protein binding capacity, tight size distribution, and stability in detergents and a variety of lysis buffer systems (pH 1 to 13). Additionally, these particles improve assay accuracy, have a high surface area per unit mass, have good lot-to-lot reproducibility, are versatile in reagent preparation, and are ready to use with no washing.

These particles are colloidally stable in the absence of a magnetic field. When a magnetic force is applied, the particles are rapidly and completely separated from suspensions.

Covalent coupling of proteins, nucleic acids, etc. to carboxyl groups on the surface is easily accomplished using our standard coupling technology. To learn more about this procedure, read our "Recommended Adsorption and Covalent Coupling Procedures" on pages 10-15 of this catalog.

Sera-Mag Magnetic Oligo(dT) Particles

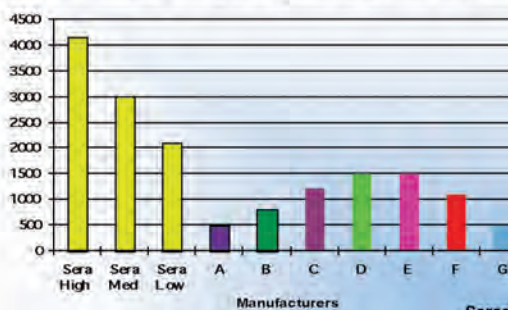
The 1 μ m Sera-Mag magnetic oligo(dT) (MG-OL) particles contain covalently bound oligo(dT)₁₄ and have an excellent shelf life stability of 48 months. The MG-OL particles are colloidally stable in the absence of a magnetic field and will remain in suspension for extended periods of time in the absence of a magnetic field. MG-OL particles are used to capture or isolate mRNA from a variety of sources.

Once isolated, further applications like RT-PCR, cDNA library construction or subtractive hybridization can be performed. The approximate mRNA binding-capacity is 11 μ g of mRNA per mg of particles (dependent upon sample and message length).

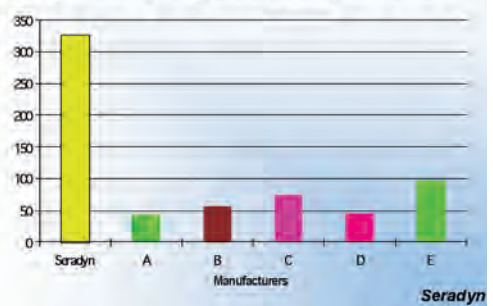
MG-OL particles can also be used as a universal base particle for coupling unique oligo sequences. Simply synthesize the oligo with a poly-A tail for easy attachment to the oligo(dT) particles.

To order Thermo Scientific Sera-Mag magnetic particles, call 1-800-232-3342 (USA) or 1-510-979-5000 (International).

Sera-Mag® Magnetic Streptavidin
Biotin Binding Capacity - Comparisons



Sera-Mag® Magnetic Oligo(dT)
(dT)₃₀ Binding Capacity Comparisons



Thermo Scientific Sera-Mag Magnetic Particles

Sera-Mag Magnetic Carboxylate-modified	Nominal Diameter	Bottle Size	Binding Capacity	Type/ Parking Area	Catalog Number
5% solids, 50 mg/mL	~1 µm	15 mL	High	Carboxyl/ PA5 / 0.05% Azide	2415-2105-050250
	~1 µm	100 mL	High	Carboxyl/ PA5 / 0.05% Azide	2415-2105-050350
	~1 µm	1000 mL	High	Carboxyl/ PA5 / 0.05% Azide	2415-2105-050450
	~1 µm	15 mL	High	Carboxyl/ PA5 / 0.05% Azide	4415-2105-050250
	~1 µm	100 mL	High	Carboxyl/ PA5 / 0.05% Azide	4415-2105-050350
	~1 µm	1000 mL	High	Carboxyl/ PA5 / 0.05% Azide	4415-2105-050450

Sera-Mag Magnetic Oligo(dT) ₁₄	Nominal Diameter	Bottle Size	Binding Capacity	Type/ Parking Area	Catalog Number
1% solids	~1 µm	1 mL	Medium	Oligo surface / 0.05% Azide	3815-2103-011150
	~1 µm	5 mL	Medium	Oligo surface / 0.05% Azide	3815-2103-010150
	~1 µm	100 mL	Medium	Oligo surface / 0.05% Azide	3815-2103-010350
Buffer Kit (mRNA isolation)		3 x 4 mL		Hybridization, wash, elution buffers	281111

Sera-Mag Magnetic Streptavidin-coated	Nominal Diameter	Bottle Size	Binding Capacity	Type/ Parking Area	Catalog Number
1% solids	~1 µm	1 mL	Low	Streptavidin level 3 / 0.05% Azide	3015-2103-011150
	~1 µm	5 mL	Low	Streptavidin level 3 / 0.05% Azide	3015-2103-010150
	~1 µm	100 mL	Low	Streptavidin level 3 / 0.05% Azide	3015-2103-010350
	~1 µm	1 mL	Medium	Streptavidin level 4 / 0.05% Azide	3015-2104-011150
	~1 µm	5 mL	Medium	Streptavidin level 4 / 0.05% Azide	3015-2104-010150
	~1 µm	100 mL	Medium	Streptavidin level 4 / 0.05% Azide	3015-2104-010350
	~1 µm	1 mL	High	Streptavidin level 5 / 0.05% Azide	3015-2105-011150
	~1 µm	5 mL	High	Streptavidin level 5 / 0.05% Azide	3015-2105-010150
	~1 µm	100 mL	High	Streptavidin level 5 / 0.05% Azide	3015-2105-010350

Level 3 = Low Binding
Level 4 = Medium Binding
Level 5 = High Binding

Sample Packs

	Package Size	Sample Pack Includes:	Catalog Number
Sera-Mag Magnetic Carboxylate-modified	2 x 15 mL	2415-2105-050250	S2444
		4415-2105-050250	
Sera-Mag Magnetic Streptavidin-coated	3 x 1 mL	3015-2103-011150 (low binding) 3015-2104-011150 (medium binding) 3015-2105-011150 (high binding)	S30345



- Core
- Magnetite
- Encapsulation

This SEM image and illustration shows the three stages of making a Sera-Mag magnetic particle. The core is the largest portion and consists of a carboxylate-modified particle. Covering the core is the magnetite. The outer layer is the encapsulation that seals the particle together.

Technical Supplement

Particle Reagent Optimization: Recommended Adsorption and Covalent Coupling Procedures

The following procedure outlines the suggested materials and process for the coupling of Thermo Scientific polymer particles to proteins. These recommended coupling procedures are designed for:

- Optimal adsorption of proteins to particles
- Optimal covalent coupling of proteins to particles
- Choice of two protocols for covalent coupling
- Simplicity, efficiency and confidence

For your reference, we offer a comprehensive laboratory manual entitled "Microparticle Reagent Optimization" that covers particle sensitization and optimization.

Principle of Protein Binding

Proteins bind to polystyrene (PS) or carboxylate-modified (CM) particles (magnetic and nonmagnetic) by adsorption.

Adsorption is mediated by hydrophobic and ionic interactions between the protein and the surface of the particles. Adsorption of proteins to particles occurs rapidly due to the particle surface free energy.

Proteins may also be covalently attached to the surface of carboxylate-modified particles. Carboxyl groups on the particles, activated by the water-soluble carbodiimide 1-ethyl-3-(3-dimethylamino) carbodiimide (EDAC), react with free amino groups of the adsorbed protein to form amide bonds.

For magnetic particles, we recommend following a covalent coupling procedure.

Performing covalent coupling with the direct EDAC procedure is universally useful. If exposure of a protein to EDAC is discovered to be harmful to the protein, then a pre-activation (active ester) step prior to introducing the protein is an alternative procedure for successful covalent coupling.

Provided here are the protocols for both adsorption and covalent coupling which have proven useful for many of our customers.

These protocols are written for 1.0 mL "optimization series" reactions. For larger reactions, all volumes may be scaled up proportionally.

Materials and Methods

1. Particles

- Polystyrene particles: Thermo Scientific polystyrene particles for immunoassays are available in standard sizes ranging from 0.05 μm to 2.0 μm . Larger particles are also available.

These polystyrene particles are manufactured by emulsion polymerization using an anionic surfactant and have surface sulfate groups which arise from the polymerization initiator.

Thermo Scientific polystyrene particles are formulated to have low free surfactant and, generally, the surfactant used does not interfere with protein binding.

For this reason, it is recommended that Thermo Scientific polystyrene particles be used without any preliminary clean-up.

- Carboxylate modified particles, non-magnetic and magnetic: Thermo Scientific carboxylate-modified particles (non-magnetic) are available in sizes ranging from 0.05 μm to 1.5 μm .

These carboxylate-modified particles are manufactured by the co-polymerization of styrene and acrylic acid using emulsion polymerization methods.

Carboxylate-modified particles are available in a wide range of carboxyl densities. Titration values in milliequivalents of carboxyl per gram of particles (mmoles/g, or $\mu\text{moles/mg}$) are provided with each lot.

In addition, the calculated parking area (area per carboxyl group) is provided with each lot.

Thermo Scientific carboxylate-modified particles are formulated to have low free detergent. The detergent used does not generally interfere with protein binding.

Carboxylate-modified particles may be rigorously cleaned by ion exchange with mixed bed resin or by tangential flow filtration (TFF).

Such cleaning removes various ionic byproducts, including detergent, soluble polymers and buffer salts, which may affect coupling chemistry.

The need for preliminary clean-up of carboxylate-modified particles should be established on a case-by-case basis.

Thermo Scientific Sera-Mag particles are available in a nominal 1 μm size and are encapsulated with a carboxylated polymer surface. The amount of acid on the surface of these particles is typically higher than our non-magnetic particles

Note: Parking area (PA) is a parameter that allows comparison of carboxylate-modified particles of different diameters and titration values (mEq/g). It is an area of normalized density of carboxyl groups, given in $\text{\AA}^2/\text{COOH}$. If two particles have the same PA, a particular protein molecule will "park on" the same number of carboxyl groups on the surface of either particle, and have an equivalent opportunity for covalent coupling (assuming all the carboxyls are activated).

2. BCA (Bicinchoninic Acid) Surface-Bound Protein Assay for particles:

See our Technical Supplement, "BCA Assay for Particles," for materials and methods, at www.thermoscientific.com/particletechnology.

3. Reaction Buffer: MES Buffer
2-(N-morpholino)ethanesulfonic acid:
Prepare 10X stock buffer at 500 mM, pH 6.1. The pH will not change significantly on dilution. Store at 4°C and discard if yellowed or contaminated.
4. EDAC 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride 52 µmol/mL:
Just before use, weigh approximately 10 mg of EDAC on an analytical balance. For each 10.0 mg weighed, add 1.0 mL of deionized water.

Note: EDAC is very sensitive to moisture and undergoes rapid hydrolysis in aqueous solutions. EDAC should be stored in a desiccator at -5°C and brought to room temperature before weighing.

5. NHS, N-hydroxysuccinamide (Active Ester-Two-step Coupling Procedure only): 50 mg/mL in water (very soluble).
6. Protein Stocks: Typically, a protein stock in the range of 1-10 mg/mL is recommended.

Note: The protein to be coated onto particles should be completely dissolved and not too concentrated.

7. DI water

Appropriate labware including:

- Pipettes and tips (10 µL – 5 mL)
- Mixing wheel or other device
- Appropriate magnetic separation device for Sera-Mag particles
- Microcentrifuge tubes
- Microcentrifuge
- Tangential flow filtration: Smaller particles may require tangential flow filtration or ultra-centrifugation for washing

Note: Tangential flow membrane devices are available from several suppliers such as Spectrum Labs in sizes suitable for processing particles in milliliter to liter quantities. Particles as small as 0.05 µm may be reliably processed with TFF membranes.

- Probe-type ultrasonicator: A probe-type ultrasonicator with a microtip should be used for resuspending particle pellets during washing.

Sonication is also helpful for re-dispersing clumped particles in a stabilizing buffer.

An immersible ultrasonic probe is the ideal tool for efficient resuspension of particle pellets. For 1.0 mL reactions, a few seconds of sonication is sufficient.

Alternatively, pellets may be stirred or resuspended by repeated aspiration with a fine pipette tip. Note: Vortex mixing and bath-type sonicators are not effective for resuspending most pellets.

Particle Adsorption

Before You Begin:

- The optimal amount of protein to use depends on several factors:
 - (1) Surface area available: surface area per mg of particle increases linearly with decreasing particle diameter.
 - (2) Colloidal stability: proteins can have stabilizing or destabilizing effects on particles.
 - (3) Immunoreactivity: the optimal amount of bound sensitizing protein must ultimately be determined by functional assay.
- When the protein is added to the particles, rapid mixing is critical for even coating.

When working at a 1 mL scale, “pipette” the protein stock directly into the buffered particles, and use the same pipette tip to “syringe” the solution (mix up and down quickly).

When working on a larger scale, put the particles in a beaker with a stir bar, mix well, and add the protein stock quickly into the middle of the vortex.

- Performing a protein titration or binding isotherm is a good first experiment.
- For a 0.3 µm diameter particle (non-magnetic), a reasonable starting range would be a 10-200 µg protein/mg particle.
- Adsorbed proteins may elute from the particle surface if the wash/storage buffers are different from the adsorption buffer.
- Many detergents will elute adsorbed proteins and should not be used with the adsorption protocol.

Procedure

1. Calculate the amount of each component needed.

Note: The Coupling Procedure MS Excel Calculation Sheet may be utilized by placing “0” in the fields for EDAC:COOH.

2. Prepare and check all stock components required.
3. Once the amount of each component is prepared, set up the binding reaction by pipetting the following into microcentrifuge tubes in the order given:
 - a. 50 µL 500 mM stock MES buffer: 25 mM final
 - b. DI water to make 1.0 mL final volume
 - c. 100 µL of 10.0% solids stock particles: 1.0% solids final
 - d. Protein stock solution: the protein should be added last and mixed very rapidly into the reaction mixture by syringing repeatedly with the pipettor

Note: Improper mixing can yield unevenly coated particles.

(Continued on page 12)

Technical Supplement: Particle Reagent Optimization

(Continued from page 11)

- Mix tubes at room temperature on a mixing wheel or other device for one hour.

Note: Gentle, constant mixing is important for particle reactions.

- Remove unbound protein: pellet particles by centrifugation and decant the supernatant.
- Perform two washes with your buffer (this may be the MES buffer). Pellet particles by centrifugation and decant the supernatant. Resuspend pellets between washes using ultrasonication.
- Resuspend final pellet to desired % solids with the same buffer. For example, if the target % solids is 1.0%, then add 0.97 mL of the same buffer, given that some liquid remains after pellet formation.
- Perform the Thermo Scientific BCA Assay for Particles procedure as an analytical tool to assess the amount of protein bound on the particles.

Covalent Coupling

Before You Begin:

- To determine the optimal amount of EDAC concentration (EDAC:COOH) in one step covalent coupling, an EDAC titration (holding the protein constant) is performed.

Note: The Coupling Procedure MS Excel Calculation Sheet may be utilized by placing ranges of concentrations in the "EDAC:COOH" fields and a constant value for the "Protein added" fields.

It is recommended to use an approximate 0.5 to 2.5 fold molar excess over particle carboxyl concentration. For Sera-Mag particles, the following ratios of EDAC:COOH are suggested for optimization: 0, 0.5, 1, 2.5, 5 and 10.

- For Active Ester (two step coupling), the concentration of EDAC:COOH may be varied. However, the recommended molar ratio is 2.5 to 1. For NHS:COOH, the recommended molar ratio is 20 to 1.

- Once an optimal EDAC concentration is determined, the optimal amount of protein to be added for meeting the application performance criteria needs to be determined. To do this, perform a protein titration holding the determined EDAC concentration fixed.

Note: The Coupling Procedure MS Excel Calculation Sheet may be utilized by placing ranges of concentrations in the "Protein added" field and the determined optimal EDAC:COOH concentration in the "EDAC:COOH" fields.

- The optimal amount of protein to use depends on several factors:
 - Surface area available: surface area per mg of particle increases linearly with decreasing particle diameter.
 - Colloidal stability: proteins can have stabilizing or destabilizing effects on particles.
 - Immunoreactivity: the optimal amount of bound sensitizing protein must ultimately be determined by functional assay.

Performing a protein titration or binding isotherm is a good first experiment. For a 0.3 μm diameter particle (non-magnetic), a reasonable starting range would be a 10-200 μg protein/mg particle. For Sera-Mag particles, we suggest starting with protein concentrations of 0, 25, 50, 75, 100 and 150 or 200 (μg /mg of particle).

- When the protein is added to the particles, rapid mixing is critical for even coating. When working at a 1 mL scale, "pipette" the protein stock directly into the buffered particles, and use the same pipette tip to "syringe" the solution (mix up and down quickly).

When working on a larger scale, put the particles in a beaker with a stir bar, mix well and add the protein stock quickly into the middle of the vortex.

- For optimization scale, it is convenient to run coupling reactions in microcentrifuge tubes. With conventional microcentrifuges such as Eppendorf, coated particles of 0.150 μm or greater diameter are pelleted in 10-30 minutes. For smaller particles of 0.150 μm or less diameter, longer centrifugation times are needed and the pellets are more difficult to resuspend.
- Smaller particles may require tangential flow filtration or ultracentrifugation for washing.
- Colloidal stability problems increase with decreasing particle diameter. Lowering the percent solids in the coupling step to 0.5% instead of 1% helps prevent clumping during coupling.
- The particles may clump during coupling due to the electrostatic effect of the positively charged EDAC molecules, the effect of the protein itself, or consumption of negative charge by amide bond formation. Washing into fresh buffer to remove EDAC and unbound protein, followed by sonication, generally reverses the clumping. Long term colloidal stability of coated particles requires development of the right storage buffer.
- The selection of storage buffer and pH is critical in achieving optimum particle performance. Zwitterionic buffers such as MOPSO, blocking proteins, and bovine serum albumin (BSA), along with fish skin gelatin (FSG), higher pH, detergents and sodium salicylate, have all proven to be useful for stabilizing particle preparations while permitting specific agglutination reactions to occur.
- Blocking proteins with a high negative charge, such as BSA and FSG, may be used to add colloidal stability, as well as block the surface against nonspecific sample adsorption. FSG works especially well with antibody-coated particles.

One Step Coupling Procedure

1. Calculate the amount of EDAC required.

Note: The "Coupling Procedure MS Excel Calculation Sheet" may be utilized to perform the calculations.

Given Equations:

(Particle acid content) mEq/g is equivalent to $\mu\text{mol}/\text{mg}$

Note: 1 mL of 1% particles contains 10 mg particles.

(Acid content, $\mu\text{mol}/\text{mg}$) (10 mg particles) (desired ratio) = μmol EDAC required

$(\mu\text{mol EDAC required})/(52 \mu\text{mol}/\text{mL}) = \text{mL EDAC stock per mL of reaction}$

2. Set up binding reaction by "pipetting" into microcentrifuge tubes in the order given:

- a. 500 mM stock MES buffer: 25 mM final
- b. Water to make 1.0 mL final volume
- c. 10.0% solids stock particles: 1.0% solids final
- d. Protein stock solution (add last)

3. Mix the tubes for approximately 15 minutes on a mixing wheel at room temperature.

Note: Gentle, constant mixing is important for particle reactions.

4. Prepare the EDAC solution immediately before use and mix the calculated volume rapidly into the reaction by syringing repeatedly with the pipettor.
5. Mix tubes at room temperature on a mixing wheel or other device for one hour. Particles may clump during this time, but this is not unusual or harmful.
6. Remove unbound protein: pellet particles by centrifugation for carboxylate-modified particles, or by magnet for Sera-Mag particles, and decant the supernatant.
7. Perform two washes with your buffer (this may be the MES buffer or a higher pH buffer of your choice). Pellet particles by centrifugation for carboxylate-modified particles, or by magnet for Sera-Mag particles, and decant the supernatant. Resuspend pellets between washes by ultrasonication.

8. Resuspend final pellet to desired percentage solids with buffer that does not contain blocking proteins. This may be the MES buffer or a higher pH buffer of your choice. For example: if the target % solids is 1.0%, then one would add 0.97 mL of the same buffer, given that some liquid remains after pellet formation.

9. Perform the Thermo Scientific BCA Assay for particles as an analytical tool to assess the amount of protein bound to the particles.

10. For long term colloidal stability, a stabilizing storage buffer will be needed. After performing the protein analysis, coated particles can be pelleted and re-suspended in a variety of storage buffers, and the colloidal stability and reactivity optimized.

Note: Covalently bound protein will not elute when subjected to detergent washes or buffer changes. As a result, covalently coupled reagents are compatible with a wider variety of buffer additives than reagents where the proteins are only adsorbed to the particles.

Active Ester Two Step Coupling Procedure

Step One: Preactivation

1. Pipette into microcentrifuge tubes in the order given:
 - a. 100 μL of 500 mM MES buffer: 50mM final
 - b. Water to make 1.0 mL final volume
 - c. 100 μL of 10.0% solids stock particles: 1.0% solids final
 - d. 230 μL NHS solution: 100 mM final
 - e. EDAC solution, calculated amount
2. Mix tubes at room temperature on a mixing wheel or other device for 30 minutes.

Note: Gentle, constant mixing is important for particle reactions.

3. Pellet particles by centrifugation for carboxylate-modified particles, or by magnet for Sera-Mag particles and decant the supernatant.

4. Resuspend particles with 1 mL 50 mM MES buffer, pH 6.1. Pellet particles by centrifugation for carboxylate-modified particles, or by magnet for Sera-Mag particles, and decant the supernatant.

5. Resuspend the pellet by adding the following and sonicating:
 - a. 100 μL 500 mM MES buffer: 50 mM final
 - b. Water to make 1.0 mL final volume

Step Two: Protein Coupling

6. Add the protein stock solution.
7. Mix tubes at room temperature on a mixing wheel or other device for 1 hour.

Note: Gentle, constant mixing is important for particle reactions.

8. Remove unbound protein: pellet particles by centrifugation for carboxylate-modified particles, or by magnet for Sera-Mag particles, and decant the supernatant.
9. Perform two washes with your 50mM buffer (this may be the MES buffer or a higher pH buffer of your choice).

Pellet the particles by centrifugation for carboxylate-modified particles, or by magnet for Sera-Mag particles, and decant the supernatant.

Resuspend pellets between washes by ultrasonication.

10. Resuspend final pellet to desired % solids with buffer that does not contain blocking proteins. (This may be the MES buffer or a higher pH buffer of your choice.)

For example: If the target % solids is 1.0%, then one would add 0.97 mL of the same buffer, given that some liquid remains after pellet formation.

11. Perform the Thermo Scientific BCA Assay for particles as an analytical tool to assess the amount of protein bound to the particles.

(Continued on page 14)

Technical Supplement: Particle Reagent Optimization

(Continued from page 13)

12. For long-term colloidal stability, a stabilizing storage buffer will be needed.

After performing the protein analysis, coated particles can be centrifuged and resuspended in a variety of storage buffers, and the colloidal stability and reactivity optimized.

Note: Covalently bound protein will not elute when subjected to detergent washes or buffer changes. As a result, covalently coupled reagents are compatible with a wider variety of buffer additives than reagents where the proteins are merely adsorbed to the particles.

Coupling Oligonucleotides Procedure

Before You Begin:

- It is good lab practice to perform this procedure in an RNase/DNase free lab environment
- If one selects a 5 mL reaction volume, then adjust to maintain % solids and concentrations, accordingly. Another option for the scale is a 1 mL build that enables one to multiply by 10 for a larger 10 mL scale.
- An oligo input loading of 2 nmol/mg (2 nmol of oligo per mg of particle) is recommended as a starting point for experimentation, but this value must be optimized to fit each particular application

Materials

1. Sera-Mag Magnetic or Sera-Mag SpeedBeads particles: Coupling reactions are typically performed at a particle concentration of 1% solids.
2. Coupling Buffer: 50 mM MES Buffer at pH 6.0.
3. Amine-Modified Oligo.

4. Coupling Reagent:

N-Ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC). The final EDAC concentration should be 1% (w/v) of the final reaction volume.

For example, add 0.5 mL of a freshly prepared 10% (w/v) EDAC solution for a 5 mL total reaction volume.

Note: EDAC is very sensitive to moisture and undergoes rapid hydrolysis in aqueous solutions. EDAC should be stored in a desiccator at -5°C and brought to room temperature before weighing.

5. Wash Buffers:

- a. 0.1 M imidazole at pH 6.0
- b. 0.1 M sodium bicarbonate buffer, native pH

Coupling Procedure

1. Vortex the stock particle suspension before use to ensure that there is no visible pellet on the bottom, or particle clumps clinging to the wall of the storage container.

Note: Upon storage, Sera-Mag SpeedBeads particles settle over time and must be completely resuspended before use.

2. In a suitable container, i.e. Falcon tube, microcentrifuge tube, HDPE bottle, etc., set up the coupling reaction by adding each of the following components in this order:

- a. RNase/DNase free water – Amount necessary to bring the reaction to desired final volume
- b. Coupling Buffer

Note: Typically, a 10x buffer stock is prepared and an aliquot of 1/10th the final reaction volume is added. For example, add 0.5 mL of 500 mM MES Buffer at pH 6.0 for a 5 mL total reaction volume.

- c. Sera-Mag SpeedBeads particles

Note: Sera-Mag Speedbeads particles are provided as a 5% stock particle suspension. To use at a 1% final particle concentration, perform a 5x dilution. For example, add 1 mL of the stock particle suspension at 5% solids to 4 mL of coupling buffer for a total reaction volume of 5 mL.

- d. Amine-Modified Oligo of choice in H₂O.

Note: Most oligo comes as a lyophilized powder. Reconstitute oligo in water before starting experiment.

- e. Freshly prepared EDAC solution.

Note: Prepare a fresh 10% (w/v) EDAC stock solution in RNase/DNase free water less than 5 minutes before use.

3. Perform the coupling reaction at 37°C overnight with continuous mixing.

Note: Use a roller or rocker (as long as the bottle has a head-to-tail orientation on the rocker). Do not use a magnetic stir bar.

4. Wash twice with 1x reaction volume of RNase/DNase free water.

Note: To perform a wash, magnetically separate the particles, aspirate the clear supernatant liquid, remove magnet and resuspend particles by vortexing in an aliquot of wash buffer equivalent to the reaction volume.

5. Wash twice with 1x reaction volume of 0.1 M imidazole (pH 6.0) at 37°C. Incubate for 5 minutes.*
6. Wash three times with 1x reaction volume of 0.1 M sodium bicarbonate at 37°C. Incubate for 5 minutes.*
7. Wash twice with 1x reaction volume of 0.1M sodium bicarbonate at 65°C. Incubate for 30 minutes.*
8. Store at 1% solids in RNase/DNase free water or an appropriate buffer for your downstream application.

*For particles less than 1 μm , no agitation is required during incubation. For particles greater than 1 μm , incubation with continuous agitation is preferred.

Reference: Benjamin B Stone and W. G. Weisburg, *Mol. and Cell. Probes*, 1996, 10, 359-370.

Coupling Procedure for Sera-Mag Blocked Amino Particles

Before You Begin:

- Coupling efficiency and particle performance is application specific. As a result, this procedure should be optimized to produce best results.

Refer to MSDS for reagents used and follow appropriate handling precautions.

- Each wash consists of magnetic separation, aspiration of supernatant, addition of buffer, and vortexing for 15 seconds (or a time sufficient to resuspend pellet). A wash step begins with each addition of buffer.

Materials

1. Sera-Mag SpeedBeads blocked amine particles: Coupling reactions are typically performed at a particle concentration of 1% solids.

2. Coupling Reagents

- 25% glutaraldehyde solution, in water, electron microscope grade
- Sodium cyanoborohydride

3. Buffer A

- 10 mM pyridine, pH 6.0

4. Buffer B

- Option 1: 10 mM pyridine, pH 6.0
- Option 2: 50 mM bicarbonate buffer at pH 10.0

5. Buffer C

- Option 1: 0.1 M ethanolamine, pH 8.0
- Option 2: 0.1 M glycine, pH 8.0

6. Storage Solutions

- Option 1: 0.05 % sodium azide
- Option 2: Buffer of choice

Coupling Procedure

1. Place desired amount of particle into a suitable container. Sera-Mag Blocked particles are supplied at 1% solids.

For a 5 mL coupling reaction volume (50 mg particle solid), place 5 mL of mixed particle suspension into the container.

2. Wash the particles twice with Buffer A. For a 5 mL reaction, add 5 mL of Buffer A to the pellet for each wash.

3. Resuspend particle pellet to 2.5% solids with Buffer A. For a 5 mL reaction, resuspend pellet to 2 mL final volume.

4. Add 10 μL of 25% glutaraldehyde solution per mg of particle (this should bring particle concentration to 2% solids, and glutaraldehyde concentration to 5% during activation).

For a 5 mL reaction, add 500 μL of 25% glutaraldehyde.

5. Mix (roll or mechanically stir) for three hours.

6. Wash particles four times with Buffer B. For a 5 mL reaction, each wash consists of 5 mL of Buffer B.

7. Resuspend final pellet with Buffer B to a concentration of 2% solids. For a 5 mL reaction, resuspend pellet with 2.5 mL of Buffer B.

8. Add protein stock solution to achieve desired input loading (20 $\mu\text{g}/\text{mg}$ to 100 $\mu\text{g}/\text{mg}$ typical input range).

For example, a 5 mL (50 mg particle solid) reaction with a desired protein input of 40 $\mu\text{g}/\text{mg}$ and a protein stock solution concentration of 20 mg/mL would require 100 μL of protein stock solution.

9. Dilute with Buffer B to achieve a final particle concentration of 1% solids. For a 5 mL reaction, dilute with Buffer B to a total final reaction volume of 5 mL.

10. Mix (roll or mechanically stir) overnight

11. Magnetically separate and remove supernatant.

12. Resuspend pellet to 2% solids with Buffer B. For a 5 mL reaction, resuspend pellet in 2.5 mL of Buffer B.

13. Prepare a 2.5% sodium cyanoborohydride stock solution in Buffer B. For a 5 mL reaction, prepare 2.5 mL of stock solution by dissolving 0.063 g of sodium cyanoborohydride in 2.44 mL of Buffer B. Mix until completely dissolved. Dispose of excess solution in accordance with local regulations.

14. Add sodium cyanoborohydride stock solution and additional Buffer B to produce 1% sodium cyanoborohydride and 1% particle solid final. For a 5 mL reaction, add 2.0 mL of sodium cyanoborohydride stock solution and 0.5 mL of Buffer B.

15. Mix (roll or mechanically stir) for one hour.

16. Add 250 μL of Buffer C per each 1 mL of reaction volume. For a 5 mL reaction, add 1.25 mL of Buffer C.

17. Continue to mix (roll or mechanically stir) for one hour.

18. Wash four times with storage solution. For a 5 mL reaction, use 5 mL of storage solution (typically 0.05 % sodium azide or any buffer of choice) for each wash.

19. Resuspend to final desired particle concentration with storage solution. For a 5 mL reaction, resuspend to 5 mL to achieve 1% solids.

View our technical notes at www.thermoscientific.com/particletechnology

Thermo Scientific Size Standards and Count Controls

NIST Traceable Size Standards

- Duke Standards - 2000 Series Uniform Particles
- Nanosphere Standards - 3000 Series Monodisperse Particles
- Duke Standards - 4000 Series Monosized Particles
- Duke Standards - 8000 Series Silica Particles
- Duke Standards - 9000 Series Glass Particles
- Chromosphere - T Certified Size Standards
- Dri-Cal Particle Size Standards
- Surf-Cal Particle Size Standards

Count Controls

- Duke Standards - 3K-4K Series Particle Counter Size Standards
- Pharm-Trol Count Precision Standards
- Validex - Count Precision Standards
- Count-Cal Count Precision Standards
- Ezy-Cal Count Precision Standards

Thermo Scientific NIST Traceable Size Standards

Validate your product performance, enhance your product development

Thermo Scientific NIST traceable size standards are designed and synthesized to be used in the development, standardization and validation of most particle counting and sizing instruments.

When these instruments are called to solve real-world analytical problems, customers depend on these particles as reference standards to validate their results.

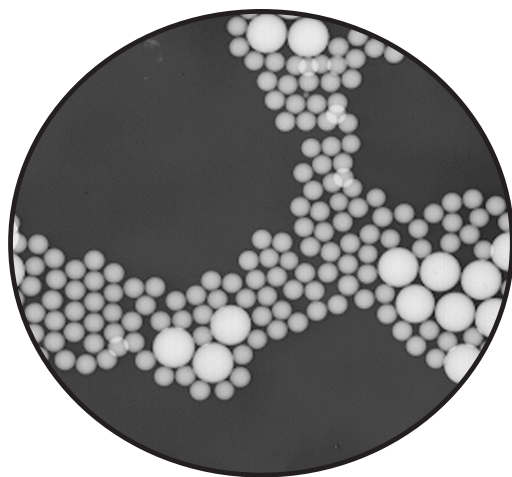
The particles are traceable to the Standard Meter through the National Institute of Standards and Technology (NIST). This enables laboratories to demonstrate the traceability of their analytical methods as required by ISO, ANSI/NCSL Z540, GMP/GLP and other standards and regulations.

The particles are also used to develop and test new analytical instruments for particle size characterization of materials.

Each package of standards contains a Certificate of Calibration and Traceability to NIST which includes a description of the calibration method and its uncertainty. It also includes a table of chemical and physical properties, and a Material Safety Data Sheet (MSDS) with handling and disposal instructions.

By using Thermo Scientific NIST traceable size standards, you have third party traceability to national and international agencies through an unbroken chain of measurements with specified uncertainties.

To order Thermo Scientific NIST traceable size standards, call 1-800-232-3342 (USA) or 1-510-979-5000 (International).



Did you know?

Pharmaceutical companies depend on Thermo Scientific NIST traceable size standards for particle contamination monitoring.

Thermo Scientific Duke Standards

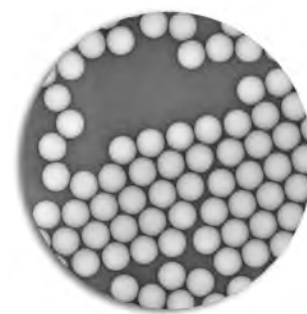
2000 Series Uniform Polymer Particles

The 2000 Series of uniform polymer particles meets the need for NIST traceable size standards that have slightly wider distributions than our monodisperse 3000 or 4000 Series size standards.

The 2000 Series is suitable for laser diffraction and other methods for analyzing wide size range materials.

The wider distribution provides light scatter across a range of detectors, resulting in a more repeatable measurement.

The material is composed of polystyrene cross-linked with divinylbenzene. This gives the particle good durability and chemical stability.



Specifications

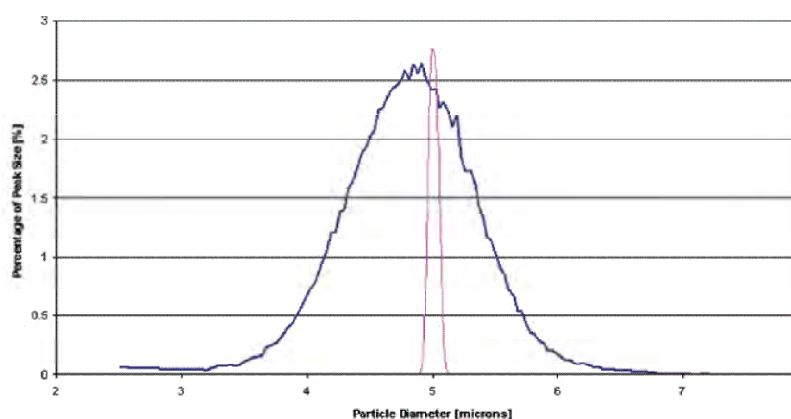
Composition: Polystyrene divinylbenzene (PS/DVB)

Density: 1.05 g/cm³

Index of Refraction: 1.59 @ 589 nm (25°C)

Additives: Contains trace amount of surfactant

Nominal Diameter	Bottle Size	% Solids	Catalog Number
Aqueous Suspensions, Calibrated by Optical Microscopy			
5 µm	15 mL	1%	2005A
6 µm	15 mL	1%	2006A
7 µm	15 mL	1%	2007A
8 µm	15 mL	1%	2008A
9 µm	15 mL	1%	2009A
10 µm	15 mL	1%	2010A
11 µm	15 mL	1%	2011A
14 µm	15 mL	1%	2014A
15 µm	15 mL	1%	2015A
20 µm	15 mL	1%	2020A
25 µm	15 mL	1%	2025A
30 µm	15 mL	1%	2030A
40 µm	15 mL	1%	2040A



This graph shows a comparison between our 4205A (see red line) and 2005A (see blue line) particles. Both particles have a nominal diameter of 5 µm, but the 2005A particle has a wider distribution of particles while our 4205A particle has a very narrow distribution. The 2000 Series standard often gives more repeatable results on some types of laser diffraction instruments.

Thermo Scientific Nanosphere Size Standards

3000 Series Monodisperse Particles

The 3000 Series of nanosphere size standards is comprised of highly uniform sulfate particles calibrated in billionths of a meter (nanometers) with NIST traceable methodology. One nanometer is 0.001 μm or 10 Angstroms.

Nanosphere size standards are ideal for the calibration of electron and atomic force microscopes.

They are also used in light scattering studies and colloidal systems research. The 20 nm to 900 nm range of diameters is convenient for checking the sizes of bacterial, viral, ribosomal and sub-cellular components.

Nanospheres are packaged as aqueous suspensions in 15 mL dropper-tipped bottles. The concentrations are optimized for ease of dispersion and colloidal stability.

Specifications

Composition:	Polystyrene
Density:	1.05 g/cm ³
Index of Refraction:	1.59 @ 589 nm (25°C)
Additives:	Contains trace amount of surfactant

Nominal Diameter	Bottle Size	% Solids	Catalog Number
Aqueous Suspensions, Calibrated by Photon Correlation Spectroscopy (PCS)			
20 nm	15 mL	1%	3020A
30 nm	15 mL	1%	3030A
40 nm	15 mL	1%	3040A
Aqueous Suspensions, Calibrated by Transmission Electron Microscopy (TEM)			
50 nm	15 mL	1%	3050A
60 nm	15 mL	1%	3060A
70 nm	15 mL	1%	3070A
80 nm	15 mL	1%	3080A
90 nm	15 mL	1%	3090A
100 nm	15 mL	1%	3100A
125 nm	15 mL	1%	3125A
150 nm	15 mL	1%	3150A
200 nm	15 mL	1%	3200A
220 nm	15 mL	1%	3220A
240 nm	15 mL	1%	3240A
270 nm	15 mL	1%	3269A
300 nm	15 mL	1%	3300A
350 nm	15 mL	1%	3350A
400 nm	15 mL	1%	3400A
450 nm	15 mL	1%	3450A
500 nm	15 mL	1%	3495A
500 nm	15 mL	1%	3500A
560 nm	15 mL	1%	3560A
600 nm	15 mL	1%	3600A
700 nm	15 mL	1%	3700A
800 nm	15 mL	1%	3800A
900 nm	15 mL	1%	3900A

Note: Due to minor variations between batches, size ranges may change slightly from batch to batch. Please visit www.thermoscientific.com/particletechnology for additional product and ordering information, or contact customer service at 1-800-232-3342 (USA) or 1-510-979-5000 (International).

Thermo Scientific Duke Standards

4000 Series Monosized Particles (Dry)

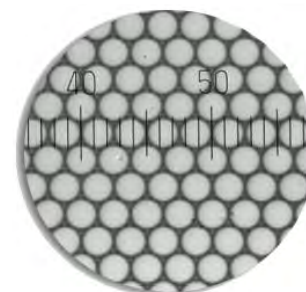
The nominal diameter of the 4000 Series of particles is calibrated with our NIST traceable microscopy methods, while the size distribution and uniformity is measured by electrical resistance analysis or optical microscopy.

Those particles with a nominal diameter from 1 µm to 160 µm are made from polystyrene, and are packaged as aqueous suspensions in 15 mL dropper-tipped bottles at an optimum concentration for dispersion, handling and dilution.

Nominal Diameter	Bottle Size	% Solids	Catalog Number
Aqueous Suspensions, Calibrated by Optical Microscopy			
1.0 µm	15 mL	1.0%	4009A
1.0 µm	15 mL	1.0%	4010A
1.1 µm	15 mL	1.0%	4011A
1.3 µm	15 mL	1.0%	4013A
1.6 µm	15 mL	1.0%	4016A
1.8 µm	15 mL	1.0%	4018A
2.0 µm	15 mL	0.4%	4202A
2.5 µm	15 mL	0.5%	4025A
3.0 µm	15 mL	0.5%	4203A
4.0 µm	15 mL	0.4%	4204A
5.0 µm	15 mL	0.3%	4205A
6.0 µm	15 mL	0.3%	4206A
7.0 µm	15 mL	0.3%	4207A
8.0 µm	15 mL	0.3%	4208A
9.0 µm	15 mL	0.3%	4209A
10 µm	15 mL	0.2%	4210A
12 µm	15 mL	0.2%	4212A
15 µm	15 mL	0.3%	4215A
20 µm	15 mL	0.3%	4220A
25 µm	15 mL	0.5%	4225A
30 µm	15 mL	0.6%	4230A
40 µm	15 mL	0.7%	4240A
50 µm	15 mL	1.4%	4250A
60 µm	15 mL	1.2%	4260A
70 µm	15 mL	2.0%	4270A
80 µm	15 mL	1.8%	4280A
100 µm	15 mL	2.1%	4310A
115 µm	15 mL	2.6%	4311A
140 µm	15 mL	4.0%	4314A
160 µm	15 mL	4.8%	4316A
Uniform Dry Spheres, Calibrated by Optical Microscopy - PSDVB			
200 µm	1 gram	2.3 x 10 ⁵ #/g	4320A
240 µm	1 gram	1.3 x 10 ⁵ #/g	4324A
280 µm	1 gram	8.3 x 10 ⁴ #/g	4328A
300 µm	1 gram	6.7 x 10 ⁴ #/g	4330A
400 µm	1 gram	2.8 x 10 ⁴ #/g	4340A
500 µm	1 gram	1.4 x 10 ⁴ #/g	4350A
550 µm	1 gram	1.1 x 10 ⁴ #/g	4355A
650 µm	1 gram	6.6 x 10 ³ #/g	4365A
Uniform Dry Spheres, Calibrated by Optical Microscopy - Polymer			
750 µm	1 gram	3.8 x 10 ³ #/g	4375A
1000 µm	1 gram	1.6 x 10 ³ #/g	4400A

Specifications

Composition: Polystyrene
 Density: 1.05 g/cm³
 Index of Refraction: 1.59 @ 589 nm (25°C)
 Additives: Trace amount of surfactant



Specifications

Composition: PSDVB
 Density: 1.05 g/cm³ (PSDVB)
 1.19 g/cm³
 Index of Refraction: 1.59 @ 589 nm (25°C)
 1.49 @ 589 nm (25°C)
 Additives: Trace amount of surfactant

Note: Products with diameters of >200 µm are packaged as dry particles. They are made from polystyrene crosslinked with divinylbenzene (PSDVB), except for the two largest products.

Thermo Scientific Duke Standards

8000 Series Silica Particles

The 8000 Series is designed for applications requiring monodisperse inorganic particles. Like glass, silica particles have a much higher density than polystyrene particles.

Because they are opaque, these particles also provide more contrast than polymer particles in optical and electron beams.

They are calibrated and certified with NIST traceable mean diameters, are suitable for a wide variety of particle measurement applications, and are packaged in pure, deionized water without any surfactants.

Specifications

Composition:	Amorphous silica
Density:	1.8 to 2.2 g/cm ³
Index of Refraction:	1.40 to 1.46 @ 589 nm (25°C)
Additives:	None

Nominal Diameter	Bottle Size	% Solids	Catalog Number
0.5 µm	15 mL	2.0%	8050
0.7 µm	15 mL	2.0%	8070
1.0 µm	15 mL	2.0%	8100
1.6 µm	15 mL	2.0%	8150



Thermo Scientific Duke Standards

9000 Series Borosilicate and Soda Lime Glass Particles (Dry)

The 9000 Series is available as uniform particles of borosilicate or soda lime glass in a range of discrete sizes from 2 µm to 2000 µm.

They also have a better tolerance to chemicals and solvents, and a higher mechanical and thermal stability.

They are calibrated and certified with NIST traceable mean diameters and are suitable for a wide variety of particle measurement applications.

The particles have been processed to remove non-spherical and broken particles.

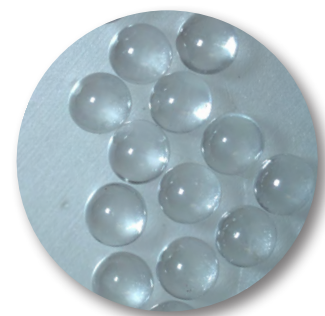
Nominal Diameter	Bottle Size	Approximate Count Per Gram	Catalog Number
Uniform Borosilicate Glass Dry Spheres - Calibrated by Optical Microscopy			
2 µm	1 gram	9.5 x 10 ¹⁰	9002
5 µm	1 gram	6.1 x 10 ⁹	9005
8 µm	1 gram	1.5 x 10 ⁹	9008
10 µm	1 gram	7.6 x 10 ⁸	9010
15 µm	1 gram	2.3 x 10 ⁸	9015
20 µm	1 gram	9.5 x 10 ⁷	9020
Uniform Soda Lime Glass Dry Spheres - Calibrated by Optical Microscopy			
30 µm	1 gram	2.8 x 10 ⁷	9030
40 µm	1 gram	1.2 x 10 ⁷	9040
50 µm	1 gram	6.1 x 10 ⁶	9050
60 µm	1 gram	3.5 x 10 ⁶	9060
70 µm	1 gram	2.2 x 10 ⁶	9070
80 µm	1 gram	1.5 x 10 ⁶	9080
90 µm	1 gram	1.0 x 10 ⁶	9090
100 µm	1 gram	7.6 x 10 ⁵	9100
110 µm	1 gram	5.7 x 10 ⁵	9110
120 µm	1 gram	4.4 x 10 ⁵	9120
140 µm	1 gram	2.8 x 10 ⁵	9140
170 µm	1 gram	1.6 x 10 ⁵	9170
200 µm	1 gram	9.5 x 10 ⁴	9200
230 µm	1 gram	6.3 x 10 ⁴	9230
280 µm	1 gram	3.5 x 10 ⁴	9280
330 µm	1 gram	2.1 x 10 ⁴	9330
400 µm	1 gram	1.2 x 10 ⁴	9400
480 µm	1 gram	6.9 x 10 ³	9480
550 µm	1 gram	4590	9550
650 µm	1 gram	2780	9650
750 µm	1 gram	1810	9750
950 µm	1 gram	890	9950
1000 µm	1 gram	760	91000
2000 µm	1 gram	95	92000

Specifications

Composition: Borosilicate glass
 Density: 2.5 - 2.55 g/cm³
 Index of Refraction: 1.56 @ 589 nm (25°C)
 Additives: None
 Packaged in: 1g, dry

Specifications

Composition: Soda lime glass
 Density: 2.4 - 2.6 g/cm³
 Index of Refraction: 1.50 - 1.52 @ 589 nm (25°C)
 Additives: None
 Packaged in: 1g, dry



Mechanical/ Electrical/ Thermal Properties

Units:	Borosilicate Glass	Soda Lime Glass
Young's Modulus [10 ⁶ psi]	10.5	10.0
Hardness [Moh]	6.5	6-7
Dielectric Constant: [22°C, 10 ⁶ Hz]	5.8	6.9
Softening Point [°C]	846	700

Note: The properties shown are typical values for bulk borosilicate and soda lime glass. They have not been measured and are not assay values for a specific batch of particles. The data is for information only and should not be used as calibration values.

Typical Composition

	Borosilicate Glass	Soda Lime Glass
SiO ₂	52.5%	60 - 72.5%
Na ₂ O	0.3%	13.7 - 17%
CaO	22.5%	9.8 - 18%
MgO	1.2%	1 - 3%
Al ₂ O ₃	14.5%	0.4 - 4%
FeO/ Fe ₂ O ₃	0.2%	0 - 0.2%
K ₂ O	0.2%	0 - 0.1%
B ₂ O ₃	8.6%	0.0%

Thermo Scientific ChromoSphere-T

Certified Size Standards - Black and Red (Dry)

ChromoSphere-T polymer particles are internally and deeply dyed with red or black dyes. The intense colors result in very high contrast and visibility relative to most background materials. They are available as dry powders and, if desired, can be easily suspended in aqueous media.

The mean diameters of ChromoSphere-T Certified Size Standards are traceable to the Standard Meter through the National Institute of Standards and Technology (NIST) and are calibrated by optical microscopy.

ChromoSphere-T Size Standards were developed for use as reference or calibration materials in applications where a high visual contrast is desired.

The particles come in a large assortment of uniform sizes between 50 μm and 500 μm in either red or black color, and are made from cross-linked polystyrene divinylbenzene (PS-DVB) copolymer (to be stored at room temperature). They can be dispersed in aqueous media with the aid of a small amount of surfactant or in lower alcohols such as methanol or ethanol.

Some dye extraction will occur when the particles are suspended in pure alcohols, but it will be minimal.

Other organic solvents, such as ethers or chlorinated hydrocarbons, should be avoided because they will swell the particles and completely extract the dye.

Specifications

Composition: Polystyrene Divinylbenzene (PS-DVB)
Density: 1.05 g/cm³
Index of Refraction: 1.59 @ 589 nm (25°C)
Additives: None

Nominal Diameter	Bottle Size	Approximate Count Per Gram	Color	Catalog Number
Dry Dyed Particles, Calibrated by Optical Microscopy				
50 μm	1 gram	1.5×10^7	Red	RD050T
50 μm	1 gram	1.5×10^7	Black	BK050T
100 μm	1 gram	1.8×10^6	Red	RD100T
100 μm	1 gram	1.8×10^6	Black	BK100T
150 μm	1 gram	5.4×10^5	Red	RD150T
150 μm	1 gram	5.4×10^5	Black	BK150T
200 μm	1 gram	2.3×10^5	Red	RD200T
200 μm	1 gram	2.3×10^5	Black	BK200T
300 μm	1 gram	6.6×10^4	Red	RD300T
300 μm	1 gram	6.6×10^4	Black	BK300T
400 μm	1 gram	2.8×10^4	Red	RD400T
400 μm	1 gram	2.8×10^4	Black	BK400T
500 μm	1 gram	1.4×10^4	Red	RD500T
500 μm	1 gram	1.4×10^4	Black	BK500T

Rinse Water Wash Studies/Particulate Decontamination Studies

Colored and fluorescent particles can be used to evaluate the cleaning capability of vial washers.

Some Helpful Tips

- The naked eye can resolve down to about 50 μm . See "ChromoSphere Polymer Particles (Dry)" on page 40
- Need even higher contrast? Fluorescent particles really stand out on a dark background. See "Fluorescent Polymer Particles (Aqueous) and Fluorescent Polymer Particles" on page 43

Important Considerations

- What is the smallest particle contaminant you are looking for? Choose a test particle that is the same size or smaller

Thermo Scientific Dri-Cal

Particle Size Standards (Dry)

Dri-Cal particle size standards are used for calibrating particle sizing and counting instrumentation that require dry particles.

These particles are conveniently packaged in dropper-tipped vials in 1 gram quantities, enabling the user to dispense the particles directly into the sampling chamber. They are not suitable for dispersion in liquid media.

Each package contains a Certificate of Calibration and Traceability to NIST which includes a description of the calibration method and its uncertainty, and a table of chemical and physical properties.

A Material Safety Data Sheet with handling and disposal instructions is also included.

Packages are lot-numbered for technical service and support after the sale.

To order Thermo Scientific Dri-Cal particle size standards, call 1-800-232-3342 (USA) or 1-510-979-5000 (International).

Specifications

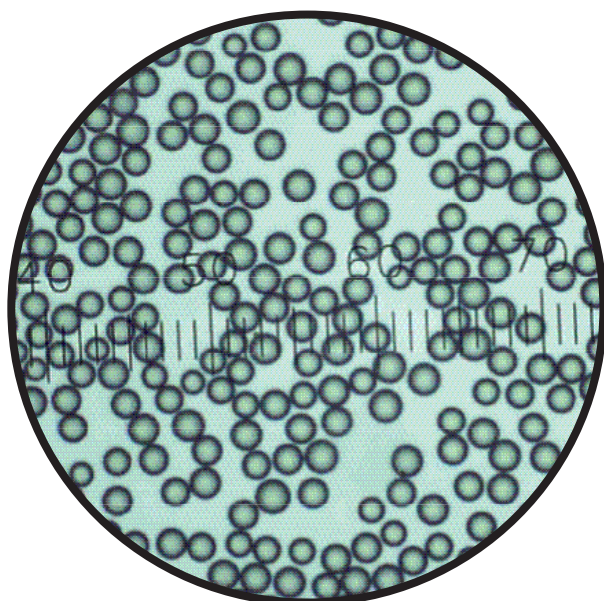
Composition: Polystyrene divinylbenzene (PS-DVB)

Density: 1.05 g/cm³

Index of Refraction: 1.59 @ 589 nm (25°C)

Additives: Trace flow agent may be present

Nominal Diameter	Bottle Size	Approximate Count Per Gram	Catalog Number
Uniform PS-DVB Dry Spheres - Calibrated by Optical Microscopy			
5 µm	1 gram	1.4 x 10 ¹⁰	DC-05
6 µm	1 gram	8.4 x 10 ⁹	DC-06
7 µm	1 gram	5.5 x 10 ⁹	DC-07
8 µm	1 gram	4.3 x 10 ⁹	DC-08
10 µm	1 gram	1.8 x 10 ⁹	DC-10
15 µm	1 gram	4.3 x 10 ⁸	DC-15
20 µm	1 gram	2.3 x 10 ⁸	DC-20
25 µm	1 gram	1.1 x 10 ⁸	DC-25
50 µm	1 gram	1.4 x 10 ⁷	DC-50
70 µm	1 gram	5.7 x 10 ⁶	DC-70
100 µm	1 gram	2.0 x 10 ⁶	DC100



Thermo Scientific Surf-Cal

Particle Size Standards

We have established specific particle sizes for use in calibrating Scanning Surface Inspection Systems (SISS) used in the semiconductor industry.

By working with instrument manufacturers, the Surf-Cal product line was created to meet SEMI standard guidelines. Available sizes include those considered to be critical sizing nodes as defined by the International Technology Roadmap for Semiconductors (ITRS)¹.

Surf-Cal is designed to simplify the job of preparing calibration wafers in your facility. Available particle sizes correspond to the calibration point sizes required by instrument manufacturers.

By depositing Surf-Cal NIST traceable polystyrene particles on specially selected wafers, you can perform periodic calibration checks and compare your scanner with scanners at other locations. You can also assess the performance of your SSIS at

critical stages in the manufacturing process.

The standards are suspended in deionized, filtered water in 50 mL bottles with a concentration of 3×10^8 particles per mL. Products PD1100 and smaller are also available at 10^{10} particles per mL for applications using the aid of a Differential Mobility Analyzer (DMA) or other size exclusionary techniques.

Measurement Methodology

To ensure direct traceability to NIST, the certified diameters of these products were transferred by transmission electron or optical microscopy from NIST standard reference materials². The uncertainty was calculated per the NIST Technical Note 1297, 1994 Edition "Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results"³. The uncertainty listed is the expanded uncertainty with a coverage factor of two (K=2).

The peak diameter was calculated using approximately the $\pm 2s$ range of the particle size distribution. The size distribution was calculated as the standard deviation of the whole peak. The Coefficient of Variation is one standard deviation expressed as a percentage of the peak diameter. The Full Weight Half Mix (FWHM) distribution was calculated as the distribution at half of the peak height expressed as a percentage of the peak diameter.

1. "The National Technology Roadmap for Semiconductors", Semiconductor Industry Association (1999)

2. S.D. Duke and E.B. Layendecker, "Internal Standard Method for Size Calibration of Sub-Micron Spherical Particles by Electron Microscopy", Fine Particle Society (1988)

3. Barry N. Taylor and Chris E. Kuyatt, "Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results". NIST Technical Note 1297, 1994 edition, September 1994.

Specifications

Composition: Polystyrene
 Density: 1.05 g/cm³
 Index of Refraction: 1.59 @ 589 nm (25°C)
 Additives: None

Certified Peak Diameter	Expanded Uncertainty (of peak diameter)	Size Distribution			Bottle Size	Catalog Number (particles per mL)	
		Std. Dev.	CV%	FWHM%		3×10^8	10^{10}
Aqueous Suspensions, Calibrated by TEM or Optical Microscopy							
0.047 μm	0.002 μm	0.004 μm	7.5%	17.4%	50 mL	PD-047	PD-047B
0.064 μm	0.002 μm	0.003 μm	5.4%	10.9%	50 mL	PD-064	PD-064B
0.083 μm	0.002 μm	0.004 μm	4.2%	9.6%	50 mL	PD-083	PD-083B
0.092 μm	0.005 μm	0.004 μm	4.6%	9.1%	50 mL	PD-092	PD-092B
0.100 μm	0.005 μm	0.004 μm	2.6%	5.2%	50 mL	PD-100	PD-100B
0.126 μm	0.006 μm	0.003 μm	2.4%	4.8%	50 mL	PD-125	PD-125B
0.155 μm	0.003 μm	0.003 μm	1.6%	3.7%	50 mL	PD-155	PD-155B
0.202 μm	0.005 μm	0.004 μm	1.8%	4.0%	50 mL	PD-200	PD-200B
0.204 μm	0.008 μm	0.004 μm	1.8%	3.7%	50 mL	PD-204	PD-204B
0.220 μm	0.007 μm	0.003 μm	1.6%	3.3%	50 mL	PD-215	PD-215B
0.304 μm	0.005 μm	0.004 μm	1.4%	3.4%	50 mL	PD-305	PD-305B
0.360 μm	0.013 μm	0.010 μm	2.0%	5.0%	50 mL	PD-365	PD-365B
0.498 μm	0.010 μm	0.006 μm	1.1%	2.5%	50 mL	PD-500	PD-500B
0.809 μm	0.014 μm	0.006 μm	0.8%	1.8%	50 mL	PD-800	PD-800B
0.802 μm	0.011 μm	0.009 μm	1.1%	2.4%	50 mL	PD-802	PD-802B
1.112 μm	0.018 μm	0.011 μm	1.0%	2.5%	50 mL	PD1100	PD1100B
1.59 μm	0.02 μm	0.016 μm	1.0%	2.6%	50 mL	PD1600	--
2.01 μm	0.04 μm	0.019 μm	1.0%	3.3%	50 mL	PD2000	--
3.04 μm	0.06 μm	0.026 μm	0.9%	2.7%	50 mL	PD3000	--

Thermo Scientific Count Controls

Thermo Scientific count controls provide an accurate and convenient method for calibrating or checking the performance of laser particle counters used in cleanrooms and other contamination monitoring applications.

The particle diameters provide third party traceability of calibration procedures to national and international agencies through an unbroken chain of measurements with specified uncertainties. The particles are suspended in a low residue diluent to minimize background interference during use.

They are available as uniform particles in a range of discrete sizes from 100 nm to 100 μm . The spherical diameters are calibrated with linear dimensions transferred from NIST Standard Reference Materials (SRM). Spheres are used instead of irregularly shaped particles to minimize the response of analytical systems to shape effects.

Each package of count controls contains a Particle Certificate of Calibration and Traceability to NIST which includes a description of the calibration method and its uncertainty, a specified particle count, and a table of chemical and physical properties.

A Material Safety Data Sheet (MSDS) with disposal instructions is also included. Packages are lot numbered for convenient technical service and support after the purchase.



Did you know?

Thermo Scientific count controls are an indispensable technology for optimizing drinking water quality.

Thermo Scientific Duke Standards

3K/4K Series - Particle Counter Size Standards

The 3K/4K Series of particles are suspensions of monodisperse polystyrene spheres designed for use in the calibration of airborne or liquid particle counting systems.

Their diameters are traceable to the Standard Meter through the National Institute of Standards and Technology (NIST).

The particles are prepared as low residue aqueous suspensions for minimal background interference.

They are also precisely diluted for immediate use in laser particle counters with minimal time-consuming adjustments of concentration.

To order Thermo Scientific Duke Standards, call 1-800-232-3342 (USA) or 1-510-979-5000 (International).

Specifications

Composition: Polystyrene

Density: 1.05 g/cm³

Index of Refraction: 1.59 @ 589 nm (25°C)

Additives: Contains trace amount of surfactant

Nominal Diameter	Bottle Size	Approximate #/mL	Catalog Number
Aqueous Suspensions, Calibrated by Transmission Electron Microscopy (TEM)			
0.1 µm	15 mL	10 ⁹	3K-100
0.15 µm	15 mL	10 ⁹	3K-150
0.2 µm	15 mL	10 ⁹	3K-200
0.22 µm	15 mL	10 ⁹	3K-220
0.27 µm	15 mL	10 ⁹	3K-269
0.3 µm	15 mL	10 ⁹	3K-300
0.35 µm	15 mL	10 ⁹	3K-350
0.4 µm	15 mL	10 ⁹	3K-400
0.5 µm	15 mL	10 ⁹	3K-500
0.6 µm	15 mL	10 ⁹	3K-600
0.7 µm	15 mL	10 ⁹	3K-700
0.8 µm	15 mL	10 ⁹	3K-800
0.9 µm	15 mL	10 ⁹	3K-900
Aqueous Suspensions, Calibrated by Optical Microscopy			
1.0 µm	15 mL	10 ⁹	3K-990
1.0 µm	15 mL	10 ⁹	3K1000
1.6 µm	15 mL	10 ⁹	3K1600
2.0 µm	15 mL	5 x 10 ⁸	4K-02
3.0 µm	15 mL	5 x 10 ⁷	4K-03
4.0 µm	15 mL	5 x 10 ⁷	4K-04
5.0 µm	15 mL	10 ⁷	4K-05
6.0 µm	15 mL	10 ⁷	4K-06
7.0 µm	15 mL	10 ⁷	4K-07
10 µm	15 mL	10 ⁶	4K-10
15 µm	15 mL	10 ⁶	4K-15
20 µm	15 mL	3 x 10 ⁵	4K-20
25 µm	15 mL	3 x 10 ⁵	4K-25
30 µm	15 mL	3 x 10 ⁵	4K-30
40 µm	15 mL	8 x 10 ⁴	4K-40
50 µm	15 mL	8 x 10 ⁴	4K-50
60 µm	15 mL	8 x 10 ⁴	4K-60
70 µm	15 mL	8 x 10 ⁴	4K-70
80 µm	15 mL	3 x 10 ⁴	4K-80
100 µm	15 mL	3 x 10 ⁴	4K100

Thermo Scientific Count Control Particles

Thermo Scientific Pharm-Trol

Pharm-Trol count controls contain NIST traceable size standards with a measured particle count. It was developed for manufacturers of parenteral drugs and ophthalmic solutions seeking interim verification of USP <788> and <789> (Particulate Matter in Injections and Particulate Matter in Ophthalmic Solutions).

These count controls are prepared with the same exacting procedures as the USP Count Reference Standard and provide process control data for particle counting consistency and verification of size calibration.

Pharm-Trol comes ready-to-use, and is designed for regular use in liquid particle counters.

As a result, Pharm-Trol enables you to document the reproducibility of the particle counter by permitting a continuous record of its performance using a particle suspension with a known concentration. The data provides the documentation needed for internal or customer quality audits.

Specifications

Composition:	Polystyrene
Density:	1.05 g/cm ³
Index of Refraction:	1.59 @ 589 nm (25°C)
Additives:	Contains trace amount of surfactant

Nominal Diameter	Bottle Size	Count/mL	Catalog Number
Aqueous Suspensions, Calibrated by Optical Microscopy			
15 µm	6 x 25 mL	3800/mL ± 15%	CS-PK
15 µm	20 X 25 mL	3800/mL ± 15%	CS-BX

Thermo Scientific Validex

Particle counting in the drinking water industry has become an indispensable method for optimizing water quality. The majority of particle counters used in the drinking water industry operate on the principle of single particle light obscuration.

Validex count controls contain NIST traceable polymer particles packaged in ultrapure water at concentrations ideal for use in validating the performance of liquid particle counters.

The composition of the suspension has been optimized to promote dispersion of the particles.

Each bottle contains 500 mL of suspension at a nominal concentration of 1000 particles per mL. A stir bar is included in the bottle to aid dispersion.

Specifications

Composition:	Polystyrene
Density:	1.05 g/cm ³
Index of Refraction:	1.59 @ 589 nm (25°C)
Additives:	Contains trace amount of surfactant

Nominal Diameter	Bottle Size	Count/mL	Catalog Number
Aqueous Suspensions, Calibrated by Optical Microscopy			
5 µm	500 mL	1000/mL ± 15%	CRS-05
10 µm	500 mL	1000/mL ± 15%	CRS-10

Thermo Scientific Count Control Particles

Thermo Scientific Count-Cal

Count-Cal count controls provide a cost effective, convenient way to validate liquid particle counters. Packaged in single-use bottles and intended to be sampled directly from the bottle, Count-Cal eliminates the need for serial dilutions and extensive sample handling, thereby minimizing contamination. It was developed for manufacturers of parenteral drugs and ophthalmic solutions seeking interim verification of USP <788> and <789> (Particulate Matter in Injections and Particulate Matter in Ophthalmic Solutions).

The particles are suspended in an ultraclean diluent and the concentration is optimized for use in light obscuration particle counters and other low concentration counting applications. The particle diameters are traceable to the Standard Meter through NIST.

Specifications

Composition: Polystyrene
 Density: 1.05 g/cm³
 Index of Refraction: 1.59 @ 589 nm (25°C)
 Additives: Contains trace amount of surfactant

Nominal Diameter	Bottle Size	Count/mL	Catalog Number
Aqueous Suspensions, Calibrated by Optical Microscopy			
2 µm	6 x 25 mL	3000/mL ± 10%	CC02-PK
5 µm	6 x 25 mL	3000/mL ± 10%	CC05-PK
10 µm	6 x 25 mL	3000/mL ± 10%	CC10-PK
15 µm	6 x 25 mL	3000/mL ± 10%	CC15-PK
20 µm	6 x 25 mL	3000/mL ± 10%	CC20-PK
25 µm	6 x 25 mL	3000/mL ± 10%	CC25-PK
30 µm	6 x 25 mL	3000/mL ± 10%	CC30-PK
50 µm	6 x 25 mL	3000/mL ± 10%	CC50-PK
70 µm	6 x 25 mL	3000/mL ± 10%	CC70-PK

Thermo Scientific Ezy-Cal

The Ezy-Cal Series of ready-to-use count controls is ideal for validating liquid particle counters. A magnetic stir bar for resuspension is included in each bottle for clean, convenient and direct sampling by instruments. The aqueous suspension medium contains a combination of dispersing agents that helps keep the particles from clumping or sticking to flow surfaces in the particle counter.

The particle diameters are traceable to the Standard Meter through NIST.

Ezy-Cal count controls are provided for setting or checking the calibration of channel thresholds or for checking the count precision of liquid borne particle counters.

Specifications

Composition: Polystyrene
 Density: 1.05 g/cm³
 Index of Refraction: 1.59 @ 589 nm (25°C)
 Additives: Contains trace amount of surfactant

Nominal Diameter	Bottle Size	Count/mL	Catalog Number
Aqueous Suspensions, Calibrated by Optical Microscopy			
2 µm	100 mL	2000/mL ± 10%	6002
5 µm	100 mL	2000/mL ± 10%	6005
10 µm	100 mL	2000/mL ± 10%	6010
15 µm	100 mL	2000/mL ± 10%	6015
20 µm	100 mL	2000/mL ± 10%	6020
25 µm	100 mL	2000/mL ± 10%	6025
30 µm	100 mL	2000/mL ± 10%	6030
50 µm	100 mL	2000/mL ± 10%	6050
70 µm	100 mL	2000/mL ± 10%	6070

Technical Supplement

Working with Particles

Particle Handling Tips

The following general guidelines provide helpful tips when using our particles and should be followed accordingly. Be sure to read the literature that accompanies your product for special handling notes (if any). If you have a critical application or are looking for a product that can be used without additional processing, please contact our technical service department.

Note: For most applications, it is imperative to ensure the cleanliness of diluents, sampling implements, and any other component that will make contact with the particles.

Resuspension

Polymer particles $\geq 0.5 \mu\text{m}$ in suspension will sediment out over time. To resuspend the particles, simply invert the bottle several times. Avoid rigorous agitation as any bubbles formed may result in statistical artifacts. Sonication after resuspension is recommended to de-gas and break up temporary agglomerates. For applications that require the particles to be suspended for an extended period of time, a clean magnetic stir bar may be used.

Dilution

Most particle suspensions are suitable for dilution and do not require additional surfactant/dispersant. However, the diluted suspensions should be used immediately as the stability may be affected.

1. Calculate the quantity of particles needed based on desired final concentration and quantity.
2. Resuspend the original particle suspension.
3. Sample immediately into clean container.
4. Add filtered deionized water to desired amount.

Suspending Dry Particles

This procedure outlines the steps necessary to put a dry powder into suspension.

1. Wet the dry particles with a 1% surfactant solution (anionic or non-ionic, i.e., Tween 20 or Triton X100) or an alcohol such as methanol or ethanol.
2. Add filtered water to the desired amount. Alternatively, let the resin settle and pour off the suspension into another clean bottle.

Drying a Suspension

Drying a suspension to achieve a dry powder is not recommended. The particles may form permanent aggregates and be aerosolized, creating an inhalation hazard.

Dissolving Polystyrene Particles

In general, aromatic hydrocarbons will dissolve polystyrene. Some commonly used solvents for this application are:

- Benzene
- Methyl ethyl ketone (MEK)
- Toluene

Note: MEK and toluene will dissolve polystyrene divinylbenzene (PSDVB) over time.

Removing/Reducing Additives By Ion Exchange Or Dialysis

These procedures are used to achieve low or surfactant-free suspensions in such applications as aerosol and biotechnology. However, removing the surfactant from a suspension may compromise the stability of the product and should be performed immediately prior to use. Please contact us if you are looking for a low or surfactant-free product.

Ion Exchange

This procedure is recommended for removing ionic surfactants from the suspension and surface of the particles:

1. Obtain mixed bed ion-exchange resin (i.e., Bio-Rad AG501-X8).
2. For a 15 mL bottle of particles at 1% solids, use three to four grams of resin.

3. Wash the resin thoroughly to remove potential contaminants.
 - a. Wash resin with approximately 200 mL deionized water five times.
 - b. Allow the resin to settle, and then pour off the water.
4. Add the particle suspension to the resin in a small bottle. Add extra water if needed.
5. Roll the mixture for four to six hours and filter through washed glass wool to remove the resin.

Dialysis

This procedure is recommended for removing surfactants from the suspension (but not from the particle surface).

1. Wash the dialysis tubing (i.e., Spectrapor 12,000-14,000 molecular weight cut-off) thoroughly with deionized water and place it in a container of deionized water (submerged).
2. Keep refrigerated for storage.
3. When ready to use, cut off the desired length of tubing.
4. Place a clamp on one end or tie it off.
5. Fill about half full with the particle suspension.
6. Clamp or tie the top end and place in the container of deionized water with at least 10 to 20 times the volume of the latex.
7. Roll or stir the contents of the container.
8. Allow to dialyze for at least four hours.
9. Repeat dialysis three times with fresh water.

View our technical notes at www.thermoscientific.com/particletechnology



Thermo Scientific Flow Cytometry Particles

Flow Cytometry Calibration and Set-Up Particles

Cyto-Cal Multifluor Fluorescence Intensity Calibrator

Cyto-Cal Alignment Particles

Cyto-Cal Count Control Particles

Flow Cytometry Multiplex Assay Particles

Cyto-Plex Carboxylated Particles

Cyto-Plex Amine Particles

Thermo Scientific Flow Cytometry Particles

For Optimizing Instrument Performance

Through optical measurements, flow cytometers distinguish cells on the basis of size and shape in addition to the presence of many different molecules inside and on the surface of the cells.

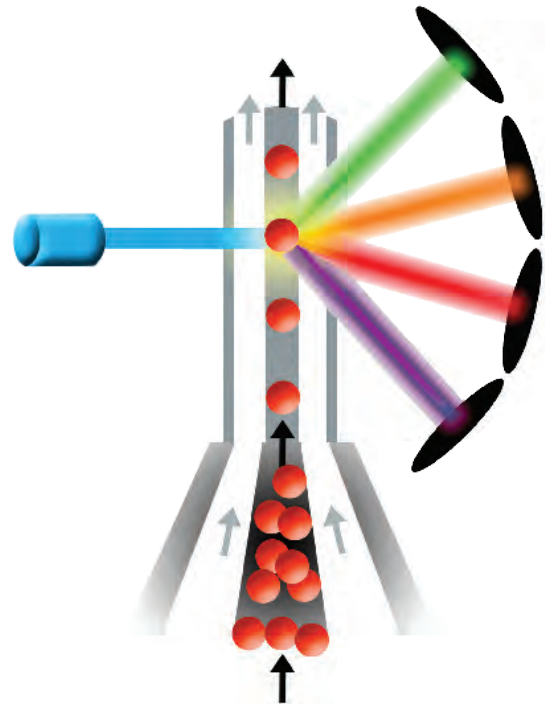
Flow cytometry is an ideal technique for counting, examining, and sorting microscopic particles suspended in a fluid stream. It allows simultaneous multiparametric analysis of the physical and/or chemical characteristics of single cells flowing through an optical and/or electronic detection apparatus.

Modern flow cytometers are able to analyze several thousand particles each second and can actively separate and isolate particles with specified properties. A flow cytometer is similar to a microscope, except that instead of producing an image of the cell, flow cytometry offers automated quantification of set parameters.

The appeal of flow cytometry arises from the flexibility and sensitivity of fluorescence technology combined with its high speed and powerful data integration capabilities.

By being a third party supplier of calibration controls, we have created an open platform of products that provide data for different instruments and laboratories without bias to any particular instrument brand.

To order Thermo Scientific flow cytometry particles, call 1-800-232-3342 (USA) or 1-510-979-5000 (International).



The above illustration shows Cyto-Cal multifluor calibration particles going through a flow cytometer. Each particle emits light in multiple channels for simultaneous detection in multiple filter sets.

Did you know?

Hospitals depend on Thermo Scientific calibration particles for monitoring CD4 counts in blood samples of AIDS patients.

Thermo Scientific Cyto-Cal

Multifluor + Violet Intensity Calibrator

The Cyto-Cal Multifluor Plus Violet Intensity Calibrator is a mixture of highly uniform 3 µm particles with green, orange, blue and red dyes in five different fluorescent intensities. It also includes one blank undyed particle for effective calibration of the fluorescent scale. These particles are so uniform that no singlet gating is required. The calibrator can monitor flow cytometer linearity while checking instrument sensitivity, stability and performance.

Packaged in an easy-to-use single-vial formula, the calibrator consists of particles precisely stained with fluorescent dyes that have optimized intensity levels and broad emissions detectable in multiple channels.

The Thermo Scientific Calicurve 1.0 software (included) provides insights into the linearity, range and calibration of the log amps to optimize flow cytometer performance.

Specifications

- Composition: Polystyrene particles containing encapsulated dyes
Dyes: Firefli fluorescent green (488/510 nm), orange (488/575 nm), red (488,633, 635/700 nm), blue (405, 450 nm)
Concentration: 1.5×10^7 particles/mL
Density: 1.06 g/cm³
Additives: 0.05% tween-20 dispersant/surfactant with 2 mM sodium azide preservative

Nominal Diameter	Bottle Size	Description	Catalog Number
	Aqueous Suspensions, Calibrated by Optical Microscopy		
~ 3 µm	2 mL (50 tests)	Visible with blue, red, violet lasers	FC3MV

Note: This product contains particles with dyes that excite and emit at the spectral ranges commonly used in flow cytometry.

488 and 633 Alignment Particles

Superior particle size and uniform dye intensity provide exceptional accuracy when performing the alignment of the flow cytometer optics to give a high level of confidence in the instrument results.

Cyto-Cal alignment particles provide a superior method for optical alignment and flow cell focusing of flow cytometers. The 3 µm particles are of the highest quality in size and fluorescence uniformity to permit the best possible optimization of each parameter being measured. Cyto-Cal alignment particles are internally dyed with chemically stable dyes and therefore have excellent signal stability.

Cyto-Cal 488 alignment particles are excited by the 488 nm spectral line of the argon laser and have broad emission, allowing them to be used to simultaneously align the FL1 (FITC), FL2 (PE) and FL3 (PE-Cy5) channels.

Cyto-Cal 633 alignment particles are optimally excited with the 633 nm He-Ne laser (635 nm diode laser) and have a maximum emission at 700 nm. They are designed to align the FL4 (APC) channel.

Specifications

- Composition: Polystyrene particles containing encapsulated dyes
Dyes: Firefli fluorescent orange (488/575 nm) or red (488,633, 635/700 nm)
Concentration: 0.5×10^6 particles/mL
Density: 1.05 g/cm³
Additives: 0.05% tween-20 dispersant/surfactant with 2 mM sodium azide preservative

Nominal Diameter	Bottle Size	Description	Catalog Number
	Aqueous Suspensions, Calibrated by Optical Microscopy		
~ 3 µm	3 mL (50 tests)	488 nm Firefli Fluorescent Orange	FA3O
~ 3 µm	3 mL (50 tests)	633 nm Firefli Fluorescent Red	FA3R

Thermo Scientific Cyto-Cal

Absolute Count Control

The Cyto-Cal absolute count control is designed for absolute cell counting on flow cytometers.

This product contains uniform 7 µm particles containing two encapsulated dyes.

The single vial contains fluorescent particles which are precisely stained with fluorescent dyes that have optimized intensity and broad emission in multiple channels (FITC, PE, PE-Cy5).

The hydrophilic particle surface eliminates doublets, ensuring an accurate count verified by analytical procedures and providing confidence in absolute counting on flow cytometers.

Specifications

- Composition: Polystyrene particles containing encapsulated dyes
- Dyes: Firefli fluorescent green (488/510 nm) and red (570/600 nm)
- Concentration: 1×10^6 particles/mL \pm 5%
- Density: 1.05 g/cm³
- Additives: 0.05% tween-20 dispersant/surfactant with 2 mM sodium azide preservative

Nominal Diameter	Bottle Size	Description	Catalog Number
	Aqueous Suspensions, Calibrated by Optical Microscopy		
~ 7 µm	10 mL	Absolute Count Control	FC7



Thermo Scientific Cyto-Plex

Carboxylated Particles - Levels 1 to 12

Cyto-Plex carboxylated particles provide 12 levels of fluorescent intensities at two distinct diameters for analysis of up to 24 different analytes. These particles consist of a highly uniform polystyrene carboxylate-modified particle with fluorescent intensities which are completely separated from each other.

The use of a single diameter particle for all dye levels saves time by requiring only the development and optimization of one particle chemistry.

Multiple diameters can be combined to increase the number of analytes measured in one test. High-density binding sites and low non-specific binding enable coupling with a wide variety of antibodies, nucleic acids and other biomolecules.

Cyto-Plex carboxylated particles have a maximum emission at 700 nm and can be excited with either 488 nm or 633 nm lasers. Emission can be collected in either the PE-Cy5 or APC channels. Since there is little or no emission in the FITC and PE channels, probes utilizing either of these dyes can be effectively used as reporters.

Specifications

- Composition: Polystyrene particles containing encapsulated dyes
- Dyes: Firefli fluorescent red
- Concentration: 0.5% solids
- Density: 1.05 g/cm³
- Additives: 0.05% tween-20 dispersant / Surfactant with 2mM sodium azide preservative

Nominal Diameter	Intensity Level	Description	Catalog Number	
			1 mL	5 mL
Aqueous Suspensions, Calibrated by Optical Microscopy				
4 µm	Level 1 (low)	Carboxylated Multiplex Fluorescent Red	FM4CR01	FM4CR01B
4 µm	Level 2	Carboxylated Multiplex Fluorescent Red	FM4CR02	FM4CR02B
4 µm	Level 3	Carboxylated Multiplex Fluorescent Red	FM4CR03	FM4CR03B
4 µm	Level 4	Carboxylated Multiplex Fluorescent Red	FM4CR04	FM4CR04B
4 µm	Level 5	Carboxylated Multiplex Fluorescent Red	FM4CR05	FM4CR05B
4 µm	Level 6	Carboxylated Multiplex Fluorescent Red	FM4CR06	FM4CR06B
4 µm	Level 7	Carboxylated Multiplex Fluorescent Red	FM4CR07	FM4CR07B
4 µm	Level 8	Carboxylated Multiplex Fluorescent Red	FM4CR08	FM4CR08B
4 µm	Level 9	Carboxylated Multiplex Fluorescent Red	FM4CR09	FM4CR09B
4 µm	Level 10	Carboxylated Multiplex Fluorescent Red	FM4CR10	FM4CR10B
4 µm	Level 11	Carboxylated Multiplex Fluorescent Red	FM4CR11	FM4CR11B
4 µm	Level 12 (high)	Carboxylated Multiplex Fluorescent Red	FM4CR12	FM4CR12B
5 µm	Level 1 (low)	Carboxylated Multiplex Fluorescent Red	FM5CR01	FM5CR01B
5 µm	Level 2	Carboxylated Multiplex Fluorescent Red	FM5CR02	FM5CR02B
5 µm	Level 3	Carboxylated Multiplex Fluorescent Red	FM5CR03	FM5CR03B
5 µm	Level 4	Carboxylated Multiplex Fluorescent Red	FM5CR04	FM5CR04B
5 µm	Level 5	Carboxylated Multiplex Fluorescent Red	FM5CR05	FM5CR05B
5 µm	Level 6	Carboxylated Multiplex Fluorescent Red	FM5CR06	FM5CR06B
5 µm	Level 7	Carboxylated Multiplex Fluorescent Red	FM5CR07	FM5CR07B
5 µm	Level 8	Carboxylated Multiplex Fluorescent Red	FM5CR08	FM5CR08B
5 µm	Level 9	Carboxylated Multiplex Fluorescent Red	FM5CR09	FM5CR09B
5 µm	Level 10	Carboxylated Multiplex Fluorescent Red	FM5CR10	FM5CR10B
5 µm	Level 11	Carboxylated Multiplex Fluorescent Red	FM5CR11	FM5CR11B
5 µm	Level 12 (high)	Carboxylated Multiplex Fluorescent Red	FM5CR12	FM5CR12B

C.V. < 2%

For 4 µm particles: Concentration approximately 1.4 x 10⁸ particles/mL

For 5 µm particles: Concentration approximately 7.3 x 10⁷ particles/mL

Thermo Scientific Cyto-Plex

Amine-Modified Particles

Cyto-Plex amine-modified particles provide 10 levels of fluorescent intensities at two distinct sizes for analysis of up to 20 different analytes.

The Cyto-Plex particles consist of a highly uniform polystyrene amine-modified particle with fluorescent intensities which are completely separated from each other.

The use of a single diameter particle for all dye levels saves time by only requiring the development and optimization of one particle chemistry.

Multiple diameters can be combined to increase the number of analytes measured in one test.

High-density binding sites and low non-specific binding enable coupling with a wide variety of antibodies, nucleic acids and other biomolecules.

Cyto-Plex amine-modified particles have a maximum emission at 700 nm and can be excited with either 488 nm or 633 nm lasers.

Emission can be collected in either the PE-Cy5 or APC channels.

Since there is little or no emission in the FITC and PE channels, probes utilizing either of these dyes can be effectively used as reporters.

Specifications

Composition:	Polystyrene particles containing encapsulated dyes
Dyes:	Firefli fluorescent red
Concentration:	0.5% solids
Density:	1.05 g/cm ³
Additives:	0.05% tween-20 dispersant/Surfactant with 2M sodium azide preservative

Nominal Diameter	Intensity Level	Description	Catalog Number	
			1 mL	5 mL
Aqueous Suspensions, Calibrated by Optical Microscopy				
4 µm	Level 1 (low)	Amine-modified Multiplex Fluorescent Red	FM4NR01	FM4NR01B
4 µm	Level 2	Amine-modified Multiplex Fluorescent Red	FM4NR02	FM4NR02B
4 µm	Level 3	Amine-modified Multiplex Fluorescent Red	FM4NR03	FM4NR03B
4 µm	Level 4	Amine-modified Multiplex Fluorescent Red	FM4NR04	FM4NR04B
4 µm	Level 5	Amine-modified Multiplex Fluorescent Red	FM4NR05	FM4NR05B
4 µm	Level 6	Amine-modified Multiplex Fluorescent Red	FM4NR06	FM4NR06B
4 µm	Level 7	Amine-modified Multiplex Fluorescent Red	FM4NR07	FM4NR07B
4 µm	Level 8	Amine-modified Multiplex Fluorescent Red	FM4NR08	FM4NR08B
4 µm	Level 9	Amine-modified Multiplex Fluorescent Red	FM4NR09	FM4NR09B
4 µm	Level 10	Amine-modified Multiplex Fluorescent Red	FM4NR10	FM4NR10B
5 µm	Level 1 (low)	Amine-modified Multiplex Fluorescent Red	FM5NR01	FM5NR01B
5 µm	Level 2	Amine-modified Multiplex Fluorescent Red	FM5NR02	FM5NR02B
5 µm	Level 3	Amine-modified Multiplex Fluorescent Red	FM5NR03	FM5NR03B
5 µm	Level 4	Amine-modified Multiplex Fluorescent Red	FM5NR04	FM5NR04B
5 µm	Level 5	Amine-modified Multiplex Fluorescent Red	FM5NR05	FM5NR05B
5 µm	Level 6	Amine-modified Multiplex Fluorescent Red	FM5NR06	FM5NR06B
5 µm	Level 7	Amine-modified Multiplex Fluorescent Red	FM5NR07	FM5NR07B
5 µm	Level 8	Amine-modified Multiplex Fluorescent Red	FM5NR08	FM5NR08B
5 µm	Level 9	Amine-modified Multiplex Fluorescent Red	FM5NR09	FM5NR09B
5 µm	Level 10	Amine-modified Multiplex Fluorescent Red	FM5NR10	FM5NR10B

C.V. < 2%

For 4 µm particles: Concentration approximately 1.4 x 10⁹ particles/mL

For 5 µm particles: Concentration approximately 7.3 x 10⁷ particles/mL

Technical Supplement

Differences in Particles for Flow Cytometry Quality Control and Quantitation

Particle standards are used in a variety of flow cytometry applications. Their selection for specific applications based on physical characteristics is discussed here.

Instrument Quality Control

Instrument quality control, which includes verification of the sensitivity and performance of the fluorescence detectors and PMTs, should be monitored frequently to ensure instrument consistency over time and to detect changes in performance that could compromise data.

The ideal product for this application would feature dyes that are thermally and photolytically stable for at least one year.

Thermo Scientific Cyto-Cal multifluor particles are hard-dyed polymer particles that utilize the Thermo Scientific Firefli process to incorporate the dye throughout the polymer matrix.

These particles are stable for at least two years.

The combination of a stable dye in a hard-dyed particle creates an ideal reference particle for those seeking long-term, inter/intra lab instrument performance monitoring. These particles will test and define the condition of the optics and flow stream and ensure the ability of the instrument to resolve different cell populations.

The dyes in Cyto-Cal multifluor particles have similar optical properties, but are not spectrally equivalent to the common dyes used in flow cytometry. Therefore, these Cyto-Cal particles should not be used to obtain absolute quantitation of fluorophores on cells.

Quantitation of Fluorescent Labeled Cells

Fluorescence quantitation is used to determine the amount of fluorophore bound per cell and, ultimately, the amount of antibody bound per cell.

This requires spectrally equivalent particle standards that feature distinct populations of particles with the same dyes used to label the cells.

These surface-dyed particles simulate dye attachment to the cell membrane. Users are then able to estimate the number of dye molecules bound per cell by comparing against a particle of known Molecules of Equivalent Soluble Fluorochrome (MESF) or Mean Equivalent Fluorochrome (MEFL) to describe the intensity level.

For years, particle calibration standards have been recommended and used to perform both fluorophore quantitation and to calibrate the response of the instrument (confirm linearity/quality control)

Surface-dyed particles have often been used for both applications. However, users must remember that all surface-dyed particles are thermally and photolytically unstable and require refrigeration. They also suffer from a short shelf life.

Both instrument manufacturers and particle vendors have perpetuated this single-particle concept.

While it may seem more convenient and economical from a user's standpoint, the use of stable calibrators for instrument quality control will provide better results and be more economical.

We recommend the use of surface-dyed particles for fluorophore quantitation (featuring the common flow cytometry dyes) and hard-dyed particles such as Thermo Scientific Cyto-Cal multifluor particles for instrument quality control. See page 33 for specific product information.

For more technical notes, visit www.thermoscientific.com/particletechnology

Did you know?

Clinical instrument manufacturers use Thermo Scientific particles to perform the primary calibration and set-up of commercially available flow cytometers.



Thermo Scientific Dyed and Fluorescent Particles

Dyed Particles

- Color-Rich Dyed Carboxylate-modified Particles
- ChromoSphere Dyed Particles

Fluorescent Particles

- Fluoro-Max Fluorescent Sulfate Particles
- Fluoro-Max Fluorescent Carboxylate-modified Particles
- Fluoro-Max Fluorescent Streptavidin-coated Particles
- Fluoro-Max Green and Red Fluorescent Particles

Thermo Scientific Dyed and Fluorescent Particles

Color-Rich Dyed and Fluoro-Max Fluorescent Particles (for lateral flow tests)

Higher intensity dyed particles provide superior test sensitivity in qualitative and quantitative lateral flow tests.

These particles are internally dyed using the Thermo Scientific Color-Rich internal dyeing method or by utilizing the proprietary Firefli fluorescent dyeing process.

Color-Rich dyeing methods provide exceptional color saturation, prevent dye leaching in aqueous media, and leave the surface free for covalent coupling and optimal immunological reactivity.

Color-Rich Dyed and Fluoro-Max fluorescent particles have been specifically designed for diagnostic lateral-flow rapid assay (membrane-based) applications.

However, these particles can also be used in other applications such as clinical diagnostics, immuno/histological studies and molecular biology.

Thermo Scientific Color-Rich Dyed (Carboxylate-Modified)

- Bind ligands without dye interference
- Dye-free surface for coupling
- High protein binding capacity
- Hydrophilic—readily adsorbs and couples proteins
- Optimize assays by controlling sensitivity, specificity and stability
- Optimized acid content
- Fast coupling and processing reactions
- Easy one-step covalent coupling protocols
- Optimized two-step coupling protocols
- Ensure reproducibility
- GMP manufacturing in our ISO certified facilities



Color-Rich Dyed particles provide maximum color and brilliance for immunologically reactive surfaces

Specifications

Composition: Polystyrene or polystyrene with copolymer grafted surface
 Surface Functionalities: Carboxylate-modified
 Dyes: Blue, red, black
 Size: 0.4 µm nominal diameter
 Uniformity: < 5% CV
 Density: 1.05 g/cm³
 Additives: None

Nominal Diameter	Bottle Size	Color	% Solids	Parking Area/ Post Process	Catalog Number
0.85 µm	15 mL	Blue	2.5%	PA100/0.05% Azide	9310-1891-020250
0.85 µm	100 mL	Blue	2.5%	PA100/0.05% Azide	9310-1891-020350
0.85 µm	1000 mL	Blue	2.5%	PA100/0.05% Azide	9310-1891-020450
0.4 µm	15 mL	Blue	4%	PA60/0.05% Azide	DB1040CA
0.4 µm	15 mL	Red	4%	PA55/0.05% Azide	DR1040CA
0.4 µm	15 mL	Black	4%	PA55/0.05% Azide	DBK1040CA
0.4 µm	100 mL	Blue	4%	PA60/0.05% Azide	DB1040CB
0.4 µm	100 mL	Red	4%	PA55/0.05% Azide	DR1040CB
0.4 µm	100 mL	Black	4%	PA55/0.05% Azide	DBK1040CB

Available visible dyes

- Blue dyed particles (DB1)
- Red dyed particles (DR1)
- Black dyed particles (DBK1)

Available fluorescent dyes

- Green fluorescent UV Excited (FG1)
- Red 600 fluorescent (FR2)
- Red 660 fluorescent (FR3)

Thermo Scientific ChromoSphere Particles

Dyed Particles for Specialized Applications (Dry)

ChromoSphere polymer particles are internally and deeply dyed with red or black dyes. These intense colors result in very high contrast and visibility relative to most background materials. They are available as dry powders and can be easily suspended in aqueous media if desired.

The product line consists of a large assortment of uniform, red or black particle sizes between 50 µm and 500 µm.

The particles are made from cross-linked polystyrene divinylbenzene (PS-DVB) copolymer and should be stored at room temperature. They can be dispersed in aqueous media with the aid of a small amount of surfactant, or in lower alcohols such as methanol or ethanol.

Minimal dye extraction will occur when the particles are suspended in pure alcohols. Other organic solvents, such as ethers or chlorinated hydrocarbons, should be avoided because they will swell the particles and completely extract the dye.

Specifications

Composition: Polystyrene divinylbenzene (PS-DVB)
 Density: 1.06 g/cm³
 Additives: None

Nominal Diameter	Mean Diameter	Bottle Size	Approximate Count per Gram	Color	% Solids	Catalog Number
Dry Dyed Particles, Calibrated by Optical Microscopy						
50 µm	49 µm	1 gram	1.6 x 10 ⁷	Red (Dry)	100%	RD050
50 µm	49 µm	1 gram	1.5 x 10 ⁷	Black (Dry)	100%	BK050
100 µm	93 µm	1 gram	2.2 x 10 ⁶	Red (Dry)	100%	RD100
100 µm	95 µm	1 gram	2.1 x 10 ⁶	Black (Dry)	100%	BK100
150 µm	149 µm	1 gram	5.5 x 10 ⁵	Red (Dry)	100%	RD150
150 µm	148 µm	1 gram	5.6 x 10 ⁵	Black (Dry)	100%	BK150
200 µm	202 µm	1 gram	2.2 x 10 ⁵	Red (Dry)	100%	RD200
200 µm	200 µm	1 gram	2.3 x 10 ⁵	Black (Dry)	100%	BK200
300 µm	301 µm	1 gram	6.6 x 10 ⁴	Red (Dry)	100%	RD300
300 µm	301 µm	1 gram	6.6 x 10 ⁴	Black (Dry)	100%	BK300
400 µm	402 µm	1 gram	2.8 x 10 ⁴	Red (Dry)	100%	RD400
400 µm	402 µm	1 gram	2.8 x 10 ⁴	Black (Dry)	100%	BK400
500 µm	500 µm	1 gram	1.4 x 10 ⁴	Red (Dry)	100%	RD500
500 µm	502 µm	1 gram	1.4 x 10 ⁴	Black (Dry)	100%	BK500

Thermo Scientific Fluoro-Max Fluorescent Particles

Carboxylate-modified and Streptavidin-coated Europium Chelate Particles

The Fluoro-Max fluorescent particles are made by dyeing OptiLink carboxylate-modified particles with europium chelate and are available in standard 0.1 μm , 0.2 μm , and 0.3 μm diameters. They are dyed internally to prevent dye leaching and to assure maximum surface immunoreactivity. These particles have been specifically designed for membrane or automated fluorometric-based applications.

With an extremely broad Stokes shift, Fluoro-Max europium chelate particles help prevent non-specific fluorescence interference. Fluoro-Max particles may be used in a variety of applications such as clinical diagnostics, immuno/histological studies and research applications.

Excitation	Emission
333 nm	613 nm

Benefits

- Bind ligands without dye interference
- Dye-free surface for coupling
- High protein binding capacity
- Readily adsorbs proteins
- Optimized acid content

Applications

- Quantitative membrane-based rapid assays
- Heterogeneous assays
- Luminescent assays
- Research applications
- Phagocytosis studies
- Cell surface markers
- Research applications
- Pore size determination

Fluoro-Max Carboxylate-modified

Packaged in 1 mL, 5 mL, 100 mL. 1% solids, 10 mg/mL

Nominal Diameter	Bottle Size	% Solids	Binding Capacity	Type/ Parking Area/ Post Process	Catalog Number
0.1 μm	1 mL	1%	High	Europium Chelate/PA50/0.05% Azide	9347-0350-011150
0.1 μm	5 mL	1%	High	Europium Chelate/PA50/0.05% Azide	9347-0350-010150
0.1 μm	100 mL	1%	High	Europium Chelate/PA50/0.05% Azide	9347-0350-010350
0.2 μm	1 mL	1%	High	Europium Chelate/PA20/0.05% Azide	9347-0520-011150
0.2 μm	5 mL	1%	High	Europium Chelate/PA20/0.05% Azide	9347-0520-010150
0.2 μm	100 mL	1%	High	Europium Chelate/PA20/0.05% Azide	9347-0520-010350
0.3 μm	1 mL	1%	High	Europium Chelate/PA20/0.05% Azide	9347-0720-011150
0.3 μm	5 mL	1%	High	Europium Chelate/PA20/0.05% Azide	9347-0720-010150
0.3 μm	100 mL	1%	High	Europium Chelate/PA20/0.05% Azide	9347-0720-010350

Fluoro-Max Streptavidin-coated

Packaged in 1 mL, 5 mL, 100 mL. 1% solids, 10 mg/mL

Nominal Diameter	Bottle Size	% Solids	Binding Capacity	Type/ Parking Area/ Post Process	Catalog Number
0.3 μm	1 mL	1%	Low	Europium Streptavidin/0.05% Azide	2947-0701-011150
0.3 μm	5 mL	1%	Low	Europium Streptavidin/0.05% Azide	2947-0701-010150

Sample Packs

	Package Size	Sample Pack Includes:	Catalog Number
Fluoro-Max Fluorescent CM-Europium Chelate	3 x 1 mL	9347-0350-011150 (0.1 μm)	S9347
		9347-0520-011150 (0.2 μm)	
		9347-0720-011150 (0.3 μm)	

Thermo Scientific Fluoro-Max Fluorescent Particles

Particulate Markers (for myocardial infarction studies)

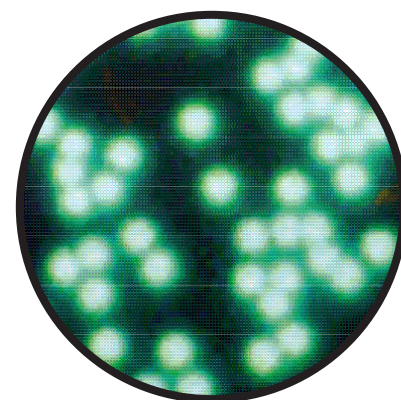
Various dyes and particulate markers have been used to mark the “risk zone” in evaluating regional ischemia. Markers that have been used include Evans Blue, India Ink and Fluorescein. However, these dyes tend to rapidly migrate throughout the tissue making the risk zone difficult to identify. Fluorescent particulate markers are effective because they lodge in the capillaries and are easily visualized under fluorescent illumination.

The fluorescent marker particles are made of polymer containing a special fluorescent dye that excites efficiently with a hand held UV lamp (i.e., Wood’s Lamp).

The fluorescence is a brilliant yellow-green color. The particles are spherical, 1-10 μm in diameter, and have a density of 1.05 g/cm^3 . This makes them easy to suspend in an aqueous medium.

The particles are heavily loaded with dye, resulting in a very strong fluorescence that can easily be seen. They are invisible under white light, allowing the non-risk tissue to be examined for infarction.

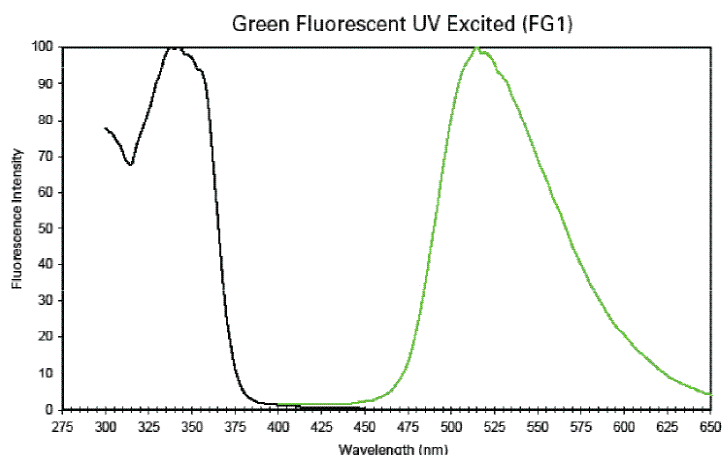
Since the dye is embedded in the interior of the particles, it does not leach out or cause indiscriminate staining.



Specifications

- Composition: Polystyrene divinylbenzene (PS-DVB)
- Dyes: Firefli fluorescent green UV (360/530 nm)
- Density: 1.05 g/cm^3
- Additives: Contains trace amount of dispersant

Nominal Diameter	Bottle Size	Fluorescent Color	% Solids	Catalog Number
Aqueous Suspensions, Calibrated by Optical Microscopy				
1 - 10 μm	1 gram	Firefli Fluorescent Green UV (Dry)	1%	34-1
1 - 10 μm	5 grams	Firefli Fluorescent Green UV (Dry)	1%	34-1B



Spectral information is approximate and for reference only. The spectral properties of the dye are dependent on their concentration and physical environment. The exact excitation and emission maxima may vary depending on the size and composition of the particles.

Thermo Scientific Fluoro-Max Fluorescent Particles

Dyed Green, Red, and Blue Aqueous (for contamination control and flow tracing)

Fluorescent particles emit bright and distinct colors when illuminated by the light of shorter wavelengths than the emission wavelength. This improves their contrast and visibility relative to background materials.

In addition to the features of conventional microscopes, the fluorescent particles offer improved sensitivity and detectability for analytical methods.

Fluorescent particles are hard-dyed (internally-dyed) polymer particles which utilize the Firefli process to incorporate the dye throughout the polymer matrix. This method produces bright fluorescent colors, minimizes photobleaching, and prevents dye leaching into aqueous media.

The particles are made of polystyrene (PS), which has a density of 1.05 g/cm³ and a refractive index of 1.59 @ 589nm (25°C). The aqueous suspensions are packaged as 1% solids.

These particles can be detected with an epifluorescence microscope, confocal microscope, fluorometer, fluorescence spectrophotometer, or fluorescence activated cell sorter. They can also be detected using mineral light or black light (UV).

Spectral information is approximate and for reference only. The spectral properties of the dyes are dependent on their concentration and physical environment.

The exact excitation and emission maxima may vary depending on the size and composition of the particles.

To order Fluoro-Max fluorescent particles, call 1-800-232-3342 (USA) or 1-510-979-5000 (International).

Specifications

Composition:	Polystyrene
Dyes	Firefli fluorescent green (468/508 nm), red (542/612 nm), blue (365, 388, 412 / 445, 445, 473 nm)
Density:	1.05 g/cm ³
Index of Refraction:	1.59 @ 589 nm (25°C)
Additives:	Contains trace amount of surfactant

Nominal Diameter	Bottle Size	Fluorescent Color	% Solids	Catalog Number
Aqueous Suspensions, Calibrated by Optical Microscopy				
0.03 µm	15 mL	Green	1%	G25
0.03 µm	90 mL	Green	1%	G25B
0.04 µm	15 mL	Green	1%	G40
0.04 µm	90 mL	Green	1%	G40B
0.05 µm	15 mL	Green	1%	G50
0.05 µm	90 mL	Green	1%	G50B
0.07 µm	15 mL	Green	1%	G75
0.07 µm	90 mL	Green	1%	G75B
0.09 µm	15 mL	Green	1%	G85
0.09 µm	90 mL	Green	1%	G85B
0.10 µm	15 mL	Green	1%	G100
0.10 µm	90 mL	Green	1%	G100B
0.14 µm	15 mL	Green	1%	G140
0.14 µm	90 mL	Green	1%	G140B
0.20 µm	15 mL	Green	1%	G200
0.20 µm	90 mL	Green	1%	G200B

More on next page...

Thermo Scientific Fluoro-Max Fluorescent Particles

Dyed Green, Red, and Blue Aqueous

Nominal Diameter	Bottle Size	Fluorescent Color	% Solids	Catalog Number
Aqueous Suspensions, Calibrated by Optical Microscopy				
0.25 µm	15 mL	Green	1%	G250
0.25 µm	90 mL	Green	1%	G250B
0.30 µm	15 mL	Green	1%	G300
0.30 µm	90 mL	Green	1%	G300B
0.40 µm	15 mL	Green	1%	G400
0.40 µm	90 mL	Green	1%	G400B
0.45 µm	15 mL	Green	1%	G450
0.45 µm	90 mL	Green	1%	G450B
0.50 µm	15 mL	Green	1%	G500
0.50 µm	90 mL	Green	1%	G500B
0.60 µm	15 mL	Green	1%	G600
0.60 µm	90 mL	Green	1%	G600B
0.70 µm	15 mL	Green	1%	G700
0.70 µm	90 mL	Green	1%	G700B
0.83 µm	15 mL	Green	1%	G830
0.83 µm	90 mL	Green	1%	G830B
0.90 µm	15 mL	Green	1%	G900
0.90 µm	90 mL	Green	1%	G900B
1 µm	10 mL	Green	1%	G0100
1 µm	60 mL	Green	1%	G0100B
2 µm	10 mL	Green	1%	G0200
2 µm	60 mL	Green	1%	G0200B
2 µm	10 mL	Green	1%	G0220
2 µm	60 mL	Green	1%	G0220B
3 µm	10 mL	Green	1%	G0300
3 µm	60 mL	Green	1%	G0300B
5 µm	10 mL	Green	1%	G0500
5 µm	60 mL	Green	1%	G0500B
10 µm	10 mL	Green	1%	G1000
10 µm	60 mL	Green	1%	G1000B
0.03 µm	15 mL	Red	1%	R25
0.03 µm	90 mL	Red	1%	R25B
0.05 µm	15 mL	Red	1%	R50
0.05 µm	90 mL	Red	1%	R50B
0.06 µm	15 mL	Red	1%	R60
0.06 µm	90 mL	Red	1%	R60B
0.10 µm	15 mL	Red	1%	R100
0.10 µm	90 mL	Red	1%	R100B
0.16 µm	15 mL	Red	1%	R160
0.16 µm	90 mL	Red	1%	R160B
0.20 µm	15 mL	Red	1%	R200
0.20 µm	90 mL	Red	1%	R200B
0.30 µm	15 mL	Red	1%	R300
0.30 µm	90 mL	Red	1%	R300B

Thermo Scientific Fluoro-Max Fluorescent Particles

Dyed Green, Red, and Blue Aqueous

Nominal Diameter	Bottle Size	Fluorescent Color	% Solids	Catalog Number
Aqueous Suspensions, Calibrated by Optical Microscopy				
0.40 µm	15 mL	Red	1%	R400
0.40 µm	90 mL	Red	1%	R400B
0.50 µm	15 mL	Red	1%	R500
0.50 µm	90 mL	Red	1%	R500B
0.60 µm	15 mL	Red	1%	R600
0.60 µm	90 mL	Red	1%	R600B
0.70 µm	15 mL	Red	1%	R700
0.70 µm	90 mL	Red	1%	R700B
0.80 µm	15 mL	Red	1%	R800
0.80 µm	90 mL	Red	1%	R800B
0.90 µm	15 mL	Red	1%	R900
0.90 µm	90 mL	Red	1%	R900B
1 µm	10 mL	Red	1%	R0100
1 µm	60 mL	Red	1%	R0100B
2 µm	10 mL	Red	1%	R0200
2 µm	60 mL	Red	1%	R0200B
3 µm	10 mL	Red	1%	R0300
3 µm	60 mL	Red	1%	R0300B
0.05 µm	15 mL	Blue	1%	B50
0.05 µm	90 mL	Blue	1%	B50B
0.10 µm	15 mL	Blue	1%	B100
0.10 µm	90 mL	Blue	1%	B100B
0.15 µm	15 mL	Blue	1%	B150
0.15 µm	90 mL	Blue	1%	B150B
0.20 µm	15 mL	Blue	1%	B200
0.20 µm	90 mL	Blue	1%	B200B
0.30 µm	15 mL	Blue	1%	B300
0.30 µm	90 mL	Blue	1%	B300B
0.40 µm	15 mL	Blue	1%	B400
0.40 µm	90 mL	Blue	1%	B400B
0.50 µm	15 mL	Blue	1%	B500
0.50 µm	90 mL	Blue	1%	B500B
0.52 µm	15 mL	Blue	1%	B520
0.52 µm	90 mL	Blue	1%	B520B
0.60 µm	15 mL	Blue	1%	B600
0.60 µm	90 mL	Blue	1%	B600B
0.70 µm	15 mL	Blue	1%	B700
0.70 µm	90 mL	Blue	1%	B700B
0.80 µm	15 mL	Blue	1%	B800
0.80 µm	90 mL	Blue	1%	B800B
0.90 µm	15 mL	Blue	1%	B900
0.90 µm	90 mL	Blue	1%	B900B
1 µm	10 mL	Blue	1%	B0100
1 µm	60 mL	Blue	1%	B0100B
2 µm	10 mL	Blue	1%	B0200
2 µm	60 mL	Blue	1%	B0200B

Thermo Scientific Fluoro-Max Fluorescent Particles

Green and Red Dry Fluorescent

Fluoro-Max fluorescent particles emit bright and distinct colors when illuminated by light of shorter wavelengths than the emission wavelength. This improves their contrast and visibility relative to background materials.

In addition to the features of conventional microscopes, these fluorescent particles offer improved sensitivity and detectability for analytical methods.

Fluorescent particles are internally dyed polymer beads which utilize the Firefli process to incorporate the dye throughout the polymer matrix.

This method produces bright fluorescent colors, minimizes photobleaching and prevents dye leaching into aqueous media.

The particles are made of polystyrene divinylbenzene (PS-DVB), which has a density of 1.05 g/cm³ and a refractive index of 1.59 @ 589nm (25°C).

These particles can be detected with an epifluorescence microscope, confocal microscope, fluorometer, fluorescence spectrophotometer, or fluorescence activated cell sorter.

They can also be detected using a mineral light or black light (UV).

Spectral information is approximate and for reference only. The spectral properties of the dyes are dependent on their concentration and physical environment.

The exact excitation and emission maxima may vary depending on the size and composition of the particles.

Specifications

Composition:	Polystyrene
Dyes:	Firefli fluorescent green (468/508 nm), red (542/612 nm)
Density:	1.05 g/cm ³
Index of Refraction:	1.59 @ 589 nm (25°C)
Additives:	Contains trace amount of surfactant

More details on next page...

Thermo Scientific Fluoro-Max Fluorescent Particles

Green and Red Dry Fluorescent

Nominal Diameter	Mean Diameter	CV% Size Uniformity	Bottle Size	Fluorescent Color	% Solids	Catalog Number
Dry Calibrated by Optical Microscopy						
5 µm	7 µm	< 18%	1 g	Green	100%	35-2
5 µm	7 µm	< 18%	5 g	Green	100%	35-2B
10 µm	8 µm	< 18%	1 g	Green	100%	35-3
10 µm	8 µm	< 18%	5 g	Green	100%	35-3B
15 µm	16 µm	< 12%	1 g	Green	100%	35-4
15 µm	16 µm	< 12%	5 g	Green	100%	35-4B
25 µm	24 µm	< 12%	1 g	Green	100%	35-5
25 µm	24 µm	< 12%	5 g	Green	100%	35-5B
30 µm	32 µm	< 13%	1 g	Green	100%	35-6
30 µm	32 µm	< 13%	5 g	Green	100%	35-6B
40 µm	39 µm	< 9%	1 g	Green	100%	35-7
40 µm	39 µm	< 9%	5 g	Green	100%	35-7B
50 µm	51 µm	< 12%	1 g	Green	100%	35-8
50 µm	51 µm	< 12%	5 g	Green	100%	35-8B
70 µm	68 µm	< 7%	1 g	Green	100%	35-9
70 µm	68 µm	< 7%	5 g	Green	100%	35-9B
80 µm	80 µm	< 6%	1 g	Green	100%	35-10
80 µm	80 µm	< 6%	5 g	Green	100%	35-10B
100 µm	90 µm	< 7%	1 g	Green	100%	35-11
100 µm	90 µm	< 7%	5 g	Green	100%	35-11B
120 µm	116 µm	< 6%	1 g	Green	100%	35-12
120 µm	116 µm	< 6%	5 g	Green	100%	35-12B
140 µm	143 µm	< 6%	1 g	Green	100%	35-13
140 µm	143 µm	< 6%	5 g	Green	100%	35-13B
160 µm	157 µm	< 5%	1 g	Green	100%	35-14
160 µm	157 µm	< 5%	5 g	Green	100%	35-14B
5 µm	7 µm	< 18%	1 g	Red	100%	36-2
5 µm	7 µm	< 18%	5 g	Red	100%	36-2B
10 µm	8 µm	< 18%	1 g	Red	100%	36-3
10 µm	8 µm	< 18%	5 g	Red	100%	36-3B
15 µm	15 µm	< 14%	1 g	Red	100%	36-4
15 µm	15 µm	< 14%	5 g	Red	100%	36-4B
25 µm	24 µm	< 12%	1 g	Red	100%	36-5
25 µm	24 µm	< 12%	5 g	Red	100%	36-5B
30 µm	31 µm	< 11%	1 g	Red	100%	36-6
30 µm	31 µm	< 11%	5 g	Red	100%	36-6B
100 µm	107 µm	< 7%	1 g	Red	100%	36-11
100 µm	107 µm	< 7%	5 g	Red	100%	36-11B

Technical Supplement

Particle Reagent Optimization - Sonication and Mixing

Introduction

Processing particles is one of the most critical phases in particle technology, and having guidance on the use of sonication will simplify your process.

For your benefit, we have new ways to utilize our particle products and services.

They are designed and engineered to meet the productivity requirements of multiple industries such as diagnostics, genomics and proteomics.

Sonication

Sonication provides a way to resuspend the particles thoroughly and efficiently without harm to the reagents. After centrifugation, processing steps and coupling reactions, difficulties that arise from improper particle resuspension can be avoided by using sonication.

We routinely sonicate our coated particle preparations with a probe-type ultrasonicator to resuspend pellets after centrifugation, and to reverse mild aggregation induced by coupling. We have not found this to be detrimental to sensitized particles in any way, and have even seen improvement in sensitivity after sonication. However, sonication may prove to be detrimental to ligand coupled particles. Therefore, we recommend vortexing slowly if sonication is not desired.

It is advisable to guard against temperature rise during sonication in sensitive systems.

Using HSA/anti-HSA as a model system, we tested whether sonication caused desorption of proteins or loss of functional activity. We subjected particle reagents to full power sonication.

Prolonged sonication did not result in measurable loss of HSA from the particle surface.

From our experience, it has proven to be virtually impossible to damage our plain sulfate and magnetic particles with sonication or heat. In certain instances, we took the plain particles to the boiling point with no ill effect. This is not true if you have ligands bound to the surface of the particle. The particle will survive, but surface ligands could be lost.

In the process of optimization of the following procedure, one should consider the characteristics of the ligand and adjust the time and handling to ensure ligand activity.

Materials

1. Probe sonicator: An immersible ultrasonic probe is the ideal tool for efficient resuspension of particle pellets. Vortex mixing and bath-type sonicators are not effective for resuspending most particle pellets.

Note: Proper performance of the sonicator is ensured when one performs appropriate maintenance of the probe.

2. Appropriate sonicator probes: A key factor that effects optimal performance of sonication is the sonication probe. The volume to be sonicated should be considered when selecting the proper probe. For example, for samples with volumes of 500 mL or less, or samples in a 1 L narrow-mouth container, we typically use a tapered micro-tip (1/8 inch diameter). For samples greater than 500 mL that are not in a narrow mouth container, we typically use a macro-tip probe (1/2 inch diameter).
3. Container for sonication: If the volume of material is 1 L or less, then the material may be sonicated in the bottle or transferred to a beaker. If a sample is larger than 1 L and in a narrow-mouth container, it needs to be transferred to an appropriate size beaker before sonication. Typically, sonication is more effective in a glass container than a plastic one.
4. Optical microscope and necessary supplies: Capable of use at 400X magnification.

Procedure

1. Handling particles before sonication: For efficiency, the material should be thoroughly mixed before the start of sonication. This is done by rolling the bottles of material using a mechanical roller or an overhead mixer for bulk material.
2. Select sonication intensity: For volumes using the micro-tip probe, the intensity is set between 30% and 40%, or a setting from 3 to 4 on a scale of 10. For volumes using the macro- tip probe, the intensity is set to 50%, or 5 on a scale of 10.

3. Select sonication time: Material being sonicated with the micro-tip probe is exposed for the following times according to fluid volume:

10 to 50 mL.....20-30 seconds

50 to 100 mL.....30-45 seconds

100 to 1000 mL ..60-90 seconds
minimum

Note: When sonicating smaller samples, the solution heats more quickly due to less volume being available to disperse the heat. For material of 10 mL or less, a vortex mixer is recommended for resuspension.

Material being sonicated with the macro tip probe is exposed for the following times according to fluid volume:

1 L.....5 minutes

3 L.....5 to 10 minutes

Greater than 3 L...Up to 20 minutes

4. Mixing particles during sonication process: It may be necessary to mix the larger samples as they sonicate, or to sonicate them using more repetitions in shorter time frames if the material tends to quickly settle out of solution. For example, do this when working with large particles greater than 1 μm , or if the material is excessively clumped:

- If sonication of material is in a 1 L bottle, the bottle with material is rolled for five minutes in between sonication increments of 10 minutes.
- If sonication of non-magnetic material is performed in a beaker, a magnetic stirrer is recommended to keep any aggregates in solution during sonication.

5. Observe dispersity of particles: After a set amount of sonication time, the material should be thoroughly mixed and observed under a microscope at 400X. When in focus, one should see a nice uniform field instead of clumps. If you see aggregates, then the material is not monodispersed. Repeat sonication and observation until you see no clumps.

- When resuspending material, visually confirm if possible that resuspension is complete by checking the bottom of the container for unsuspended material.
- Magnetic material can be mixed using all of the above methods except for the magnetic stirrers. Rolling and/or mixing times are the same for the magnetic particles as they are for any other particles of similar size and percent solids.

Mixing by Roller

Roller Mixer

The roller mixer has a motor-driven horizontal cylinder adjacent to a free-turning horizontal cylinder that together forms a cradle on which containers of product can be placed.

The placement of the free-turning cylinder can be adjusted to accommodate different sized containers.

Use a roller mixer of sufficient size and speed for the container being mixed. The roller for smaller bottles such as 100 mL can be found in small lab supply catalogs.

Mixing Time

Since the speed of the mixer is constant, mixing time is the way to control sufficient mixing. Mixing time can also vary based on the diameter of the container.

Since small diameter containers rotate faster than large diameter containers, mixing is accomplished more quickly.

Mixing time can also vary on the size of the particles. Larger particles may take more time to resuspend. Higher concentrations of particles also require more mixing time.

Note: Containers must be at least 50% and less than 90% full to have enough material covering the bottom of the container when rolling, yet not too full to prevent insufficient mixing. Extending the mixing time is acceptable. However, nothing needs to mix longer than 72 hours.

Table 1 provides guidelines for minimum mixing time according to particle and container size:

Table 1. Minimum Mixing Times Using the Roller Mixer

Container Size	Particle Size (μm)	
	$\leq 0.4\mu\text{m}$	> 0.4
≤ 1 L	10 min	40 min
>1 L	30 min	60 min

Mixing by Vortex

Vortex Mixer

A vortex mixer is used for mixing small volumes of 10 mL or less by holding the container of solution in a rubber holder and allowing a motor to rotate the shaft in an oscillating motion that causes the solution to be mixed.

Different vortex mixer models have different methods of being activated. Most have a continuous action and a manual pressure activated system. The continuous mode is generally preferred for longer vortexing times while the manual pressure mode is preferred for shorter mixing times. A vortex mixer with adjustable speed setting is recommended.

Mixing Speed

When using the controller on the mixer, adjust the speed of the mixer to a speed sufficient to cause good mixing (usually around 80% of full speed). Going too fast makes the container difficult to control.

Mixing Time

Mixing usually can be completed in 30 seconds. However, larger particles such as 0.8 and 1.0 μm require longer mixing of at least one minute or longer to resuspend, especially if the product has been stored for an extended period of time.

Verification of Mixing

Verify that the mixing is completed by observing the product during mixing to ensure adequate agitation. After mixing, make sure no product remains settled on the bottom of the container. Clumps should not be observed in the suspension under a microscope at 400X.

(Continued on page 50)

Mixing

When handling particles it is best to mix the material to ensure it is monodispersed and uniformly distributed.

Particles may be mixed according to the type of particle and volumes by the use of various equipment, including an overhead mixer, magnetic stirrer, vortex mixer and roller mixer.

An overhead mixer is typically used for pooling, diluting and handling large batches.

A vortex mixer can be used for mixing product stored in small containers such as 15 mL bottles, or other applications where the container is a similar size.

The roller mixer is used to resuspend, if necessary, and uniformly mix the particles. Magnetic stirrers are used for the purpose of making a uniform mixture rather than for resuspending.

Before Starting

- If higher than normal levels of surfactant are in the solution or if excessive foaming is observed in any of the mixing techniques, reduce the speed and time of mixing accordingly to minimize the impact on the product.

Technical Supplement: Particle Reagent Optimization

(Continued from page 49)

Mixing by Overhead Mixer

Overhead Mixer

An overhead mixer consists of a speed controllable, electrical or air-driven motor with an agitator blade and shaft attached. Choose an overhead mixer with sufficient capability to mix the volume as required. The range of volumes is dependent on the proper agitator (i.e., a short-shafted agitator for smaller volumes).

- Be sure that the container is such that the blade will be covered with enough product to prevent splashing
- Position the blade high enough on a stand to allow clearance of the container (but not so high as to prevent sufficient submersion of the agitator). Best results are usually obtained when the agitator blade can be placed at a position in the lower third of the container

Mixing Speed

The proper mixing speed can be determined by observing the action of the solution. If there is no visible movement of the product, increase the speed of the mixer until there is visible movement.

In most circumstances, overhead stirring is used to achieve or maintain a uniform mixture, therefore mixing speed is not critical as long as sufficient motion is maintained.

If one is removing aliquots from the mixture, then carefully monitor the level of product being mixed and periodically reduce the speed of the mixing to keep the product from splashing on the side of the container as the volume changes.

Mixing Time

If mixing is for the purpose of resuspension, then follow the guidelines in Table 1-Minimum Mixing Times Using the Roller Mixer.

Mixing by Magnetic Stirrer

Magnetic Stirrer

A magnetic stirrer consists of a variable speed motor with an attached magnetic rotor encased in a platform.

The rotor causes a magnetic stir bar placed in the solution to spin and mix the solution.

Select a stirrer with sufficient power to move the volume of solution and hold the container on the stirrer.

Note: Magnetic stirrers are not used for mixing magnetic particles.

- Effective mixing requires matching the container size with the volume of the solution and selecting a suitable stir bar large enough to effectively move the solution but not so large as to cause splashing. The size of the container is determined by the size of the batch and taking several factors into account. Too large a container can cause splashing and loss of yield due to increased surface area. Containers should have a volumetric working range of 20 to 80%, and have a flat bottom that allows the stir bar to spin freely
- Select an appropriate sized magnetic stir bar that will fit the container and thoroughly move the volume when stirred

Mixing Speed

Adjust the stirring speed to create enough movement of the suspension for it to be adequately mixed.

Sufficient movement ranges from creating a "dimple" 1/4-inch into the surface to a funnel shape extending approximately one-fourth of the way into the suspension.

Because the mixing is intended to evenly distribute the material in the suspension, it is not necessary to rapidly mix the suspension. However, slightly faster mixing could be required for large particles.

Avoid splashing the material. If the volume decreases, then mixing should decrease. Simply slow the speed of the mixing as the volume decreases to reduce splashing.

Mixing Time

The length of time for mixing will vary with the size of the batch, i.e., the larger the batch the longer the mixing time.

However, mixing should not take longer than 30 minutes unless resuspension is the purpose of the mixing.

If mixing is for resuspension, then follow Table 1-Minimum Mixing Times Using the Roller Mixer.

View our technical notes at www.thermoscientific.com/particletechnology



Thermo Scientific Clinical Diagnostic and Specialty Application Particles

Polymer Particles

Opti-Bind Sulfate Particles

Opti-Link Carboxylate-Modified Particles

Power-Bind Streptavidin-Coated Particles

5000 Series - Polymer Particle Suspensions

7000 Series - Copolymer Particle Suspensions

Cleanroom Particles

Smoke-Check Smoke Detector Challenge Particles

Thermo Scientific Opti-Bind and Opti-Link Particles

Opti-Bind

Sulfate Particles

Opti-Bind sulfate particles can be used directly from the bottle without any pre-cleaning for most applications. Our production does not utilize common surfactants like SDS, Tween 20, and Triton X-100 that can interfere with protein binding to particle surfaces. Additionally, our proprietary anionic surfactant does not interfere with the binding of proteins, nor cause proteins to desorb from particle surfaces.

Opti-Bind particles are available in a wide range of diameters from 0.1 μm to 2.5 μm for use in a variety of applications including turbidimetric assays.

Sulfate surfaces are very hydrophobic and adsorb proteins almost instantaneously. Opti-Bind particles have been optimized for maximum reactivity in many diagnostic applications.

To order Thermo Scientific Opti-Bind products, call 1-800-232-3342 (USA) or 1-510-979-5000 (International).

Opti-Link

Carboxylate-Modified Particles

Opti-Link carboxylate-modified (CM) particles contain carboxylic acid groups for covalent coupling and can be used in a variety of applications. The various acid content available within the Opti-Link product line enables you to control such important parameters as sensitivity, specificity and stability.

For most applications, Opti-Link particles can be used directly from the bottle without any pre-cleaning.

Our proprietary anionic surfactant does not interfere with the binding of proteins, nor cause proteins to desorb from particle surfaces.

Various surface acid concentrations are available to help optimize reagent development efforts.

To order Thermo Scientific Opti-Link products, call 1-800-232-3342 (USA) or 1-510-979-5000 (International).



From clinical immunoassays and molecular biology sample preparation to research applications, Thermo Scientific particles are critical components for many of the world's leading diagnostic and molecular biology companies.

Thermo Scientific Opti-Bind and Opti-Link Particles

Opti-Bind Sulfate Particles	Nominal Diameter	Bottle Size	% Solids	Surface Acid Loading/ Post Process	Catalog Number
100 mg/mL	0.1 µm	15 mL	10%	Low SO4/Pasteurized	8100-0397-100290
	0.1 µm	100 mL	10%	Low SO4/Pasteurized	8100-0397-100390
	0.2 µm	15 mL	10%	Low SO4/Pasteurized	8100-0597-100290
	0.2 µm	100 mL	10%	Low SO4/Pasteurized	8100-0597-100390
	0.3 µm	15 mL	10%	Low SO4/Pasteurized	8100-0797-100290
	0.3 µm	100 mL	10%	Low SO4/Pasteurized	8100-0797-100390
	0.4 µm	15 mL	10%	Low SO4/Pasteurized	8100-0997-100290
	0.4 µm	100 mL	10%	Low SO4/Pasteurized	8100-0997-100390
	0.6 µm	15 mL	10%	Low SO4/Pasteurized	9100-1397-100290
	0.6 µm	100 mL	10%	Low SO4/Pasteurized	9100-1397-100390
	0.85 µm	15 mL	10%	Low SO4/Pasteurized	9100-1897-100290
	0.85 µm	100 mL	10%	Low SO4/Pasteurized	9100-1897-100390
	1.25 µm	15 mL	10%	Low SO4/Pasteurized	7100-2697-100250
	1.25 µm	100 mL	10%	Low SO4/Pasteurized	7100-2697-100350
	2.5 µm	15 mL	10%	Low SO4/0.05% Azide	7100-3497-100250
	2.5 µm	100 mL	10%	Low SO4/0.05% Azide	7100-3497-100350
Opti-Link Carboxylate-modified	Nominal Diameter	Bottle Size	% Solids	Surface Acid Loading	Catalog Number
100 mg/mL	0.04 µm	15 mL	4%	Low Acid/Azide	W004CA
	0.04 µm	100 mL	4%	Low Acid/Azide	W004CB
	0.2 µm	15 mL	10%	Low Acid/Pasteurized	9300-0570-100290
	0.2 µm	100 mL	10%	Low Acid/Pasteurized	9300-0570-100390
	0.2 µm	15 mL	10%	Medium Acid/Pasteurized	8300-0550-100290
	0.2 µm	100 mL	10%	Medium Acid/Pasteurized	8300-0550-100390
	0.2 µm	15 mL	10%	High Acid/Pasteurized	8300-0520-100290
	0.2 µm	100 mL	10%	High Acid/Pasteurized	8300-0520-100390
	0.3 µm	15 mL	10%	Medium Acid/Pasteurized	8300-0750-100290
	0.3 µm	100 mL	10%	Medium Acid/Pasteurized	8300-0750-100390
	0.3 µm	15 mL	10%	High Acid/Pasteurized	8300-0720-100290
	0.3 µm	100 mL	10%	High Acid/Pasteurized	8300-0720-100390

More on next page...

Thermo Scientific Opti-Bind and Opti-Link Particles

Continued from prior page

Opti-Link Carboxylate-modified	Nominal Diameter	Bottle Size	% Solids	Surface Acid Loading	Catalog Number
	0.4 µm	15 mL	10%	Low Acid/Pasteurized	8300-0970-100290
	0.4 µm	100 mL	10%	Low Acid/Pasteurized	8300-0970-100390
	0.4 µm	15 mL	10%	High Acid/Pasteurized	8300-0920-100290
	0.4 µm	100 mL	10%	High Acid/Pasteurized	8300-0920-100390
	0.5 µm	15 mL	4%	Medium Acid/Azide	W050CA
	0.5 µm	100 mL	4%	Medium Acid/Azide	W050CB
	0.85 µm	15 mL	4%	Medium Acid/Azide	W080CA
	0.85 µm	100 mL	4%	Medium Acid/Azide	W080CB
	0.85 µm	15 mL	10%	Low Acid/Pasteurized	9300-1891-100290
	0.85 µm	100 mL	10%	Low Acid/Pasteurized	9300-1891-100390
	0.9 µm	15 mL	4%	High Acid/Azide	W090CA
	0.9 µm	100 mL	4%	High Acid/Azide	W090CB
	2.0 µm	15 mL	10%	PA5, High Acid	7300-3305-100250
	2.0 µm	100 mL	10%	PA5, High Acid	7300-3305-100350
	3.0 µm	15 mL	10%	PA20, High Acid	7300-3420-100250
	3.0 µm	100 mL	10%	PA20, High Acid	7300-3420-100350
	4.0 µm	15 mL	4%	High Acid/Azide	W400CA
	4.0 µm	100 mL	4%	High Acid/Azide	W400CB
	5.0 µm	15 mL	4%	High Acid/Azide	W500CA
	5.0 µm	100 mL	4%	High Acid/Azide	W500CB



Thermo Scientific Power-Bind Particles

Non-Magnetic, Streptavidin-Coated

Benefits

- Dissociation constant (K_d 10^{-15} Molar)
- Stable binding of ligands
- Easy one-step binding protocols for biotinylated ligands
- High activity of surface bound ligands
- Easily solve difficult coupling problems
- Simple aqueous biotinylation reactions
- Low non-specific interactions
- Highly mobile particles for membrane-based applications
- Can be used in EIA formats with biotin/enzyme detection systems
- Beneficial spacer effect of SA molecule
- Choice of 0.3 μm or 0.8 μm diameters for different application requirements
- High biotin-binding capacity for molecular biology applications

Power-Bind non-magnetic streptavidin-coated particles improve and simplify the binding of ligands to particles. They combine the advantages of a high surface area along with easy, high affinity and high specific activity binding. They can also be used in a variety of diagnostic and molecular biology applications.

These monodisperse particle suspensions feature streptavidin covalently bound to the surface of Opti-Link carboxylate-modified particles in a highly active form, resulting in high biotin-binding capacity and long shelf life.

The use of particles as a solid phase support in various immunoassays and affinity purifications is well known.

However, achieving high activity and stable binding of solid phase ligands has been a major challenge. Biotinylated compounds easily bind with utmost stability to Power-Bind streptavidin particles after simple incubation in buffer. This is due to the high affinity of the biotin-streptavidin interaction.

Streptavidin also functions as a spacer, which improves the specific activity of the bound ligand.

Power-Bind streptavidin particles address the concerns of diagnostic test kit manufacturers and nucleic acid researchers.

With well-known biotin-streptavidin reactions, scientists can now easily bind ligands to particle surfaces.

Compounds that are difficult to attach to particle surfaces by conventional means may be amenable to biotinylation. This includes compounds for which activation reactions must be performed in an organic solvent. In this case, biotinylation may be carried out in an organic solvent. Then, the biotin derivative is simply mixed with the Power-Bind streptavidin particles.

Nucleic acids, which adsorb poorly to particle surfaces, are readily bound to Power-Bind streptavidin particles after biotinylation.

To order Thermo Scientific Power-Bind particles, call 1-800-232-3342 (USA) or 1-510-979-5000 (International).

Power-Bind Streptavidin	Nominal Diameter	Bottle Size	% Solids	Binding Capacity	Catalog Number
	0.3 μm	1 mL	1%	~1000 pmol/mg	2900-0701-011150
	0.3 μm	5 mL	1%	~1000 pmol/mg	2900-0701-010150
	0.3 μm	100 mL	1%	~1000 pmol/mg	2900-0701-010350
	0.85 μm	1 mL	1%	~1000 pmol/mg	2900-1801-011150
	0.85 μm	5 mL	1%	~1000 pmol/mg	2900-1801-010150
	0.85 μm	100 mL	1%	~1000 pmol/mg	2900-1801-010350

Thermo Scientific 5000 Series

Polymer Particle Suspensions

The 5000 Series of polymer particle suspensions meets the need for particulate materials with a variety of sizes and properties.

They are useful for such applications as filter evaluation, checking and testing, light scattering research, fluid mechanics research, aerosol particle generation, dispersion studies, and many other research and development projects.

They are not intended for use in instrument calibration or diagnostic reagents because they lack the exacting specifications needed for these applications.

The polystyrene particles have a density of 1.05 g/cm³ and a refractive index of 1.59 @ 589 nm. Particle diameters are measured by optical microscopy, photon correlation spectroscopy or light scattering. They are packaged as aqueous suspensions at 10% solids by weight.

The 5000 Series is available in sizes from 0.03 to 3.2µm in size. If larger particles are required, please refer to our 7000 Series copolymer particle suspensions on page 57 of this catalog.

Note: 15 mL ("A" bottles) listed are available for immediate purchase. 100 mL ("B" bottles) and 1000 mL ("C" bottles) are packaged to order; i.e., 5003B and 5003C.

To order Thermo Scientific 5000 Series particles, call 1-800-232-3342 (USA) or 1-510-979-5000 (International).

Specifications

Composition: Polystyrene
 Density: 1.05 g/cm³
 Index of Refraction: 1.59 @ 589 nm (25°C)
 Additives: Contains trace amount of surfactant

Nominal Diameter	Size Uniformity	Bottle Size	% Solids	Catalog Number
0.03 µm	≤ 30%	15 mL	10%	5003A
0.06 µm	≤ 18%	15 mL	10%	5006A
0.08 µm	≤ 18%	15 mL	10%	5008A
0.09 µm	≤ 15%	15 mL	10%	5009A
0.10 µm	≤ 15%	15 mL	10%	5010A
0.11 µm	≤ 12%	15 mL	10%	5011A
0.12 µm	≤ 12%	15 mL	10%	5012A
0.14 µm	≤ 6%	15 mL	10%	5014A
0.16 µm	≤ 6%	15 mL	10%	5016A
0.17 µm	≤ 5%	15 mL	10%	5017A
0.20 µm	≤ 5%	15 mL	10%	5020A
0.22 µm	≤ 3%	15 mL	10%	5022A
0.24 µm	≤ 3%	15 mL	10%	5024A
0.26 µm	≤ 3%	15 mL	10%	5026A
0.30 µm	≤ 3%	15 mL	10%	5030A
0.31 µm	≤ 3%	15 mL	10%	5031A
0.33 µm	≤ 3%	15 mL	10%	5033A
0.36 µm	≤ 3%	15 mL	10%	5036A
0.43 µm	≤ 3%	15 mL	10%	5043A
0.45 µm	≤ 3%	15 mL	10%	5045A
0.49 µm	≤ 3%	15 mL	10%	5049A
0.50 µm	≤ 3%	15 mL	10%	5050A
0.51 µm	≤ 3%	15 mL	10%	5051A
0.52 µm	≤ 3%	15 mL	10%	5052A
0.60 µm	≤ 3%	15 mL	10%	5060A
0.65 µm	≤ 3%	15 mL	10%	5065A
0.67 µm	≤ 3%	15 mL	10%	5067A
0.81 µm	≤ 3%	15 mL	10%	5081A
0.88 µm	≤ 3%	15 mL	10%	5088A
0.93 µm	≤ 3%	15 mL	10%	5093A
1.0 µm	≤ 3%	15 mL	10%	5100A
1.3 µm	≤ 5%	15 mL	10%	5130A
1.5 µm	≤ 4%	15 mL	10%	5153A
2.0 µm	≤ 4%	15 mL	10%	5200A
2.9 µm	≤ 5%	15 mL	10%	5300A
3.2 µm	≤ 5%	15 mL	10%	5320A

Thermo Scientific 7000 Series

Copolymer Particle Suspensions

Suspensions of large copolymer particles are useful as model systems for fluid mechanics experiments and as challenge particles for large pore filtration systems.

These particles are designed to meet the need for particulate materials with a variety of sizes and properties. They are useful for such applications as filter evaluation, filter testing, fluid mechanics research, dispersion studies, and many other research and development projects.

They are not intended for use in instrument calibration or diagnostic reagents because they lack the exacting specifications needed for these applications.

The 7000 Series is also useful as experimental particles for acoustical and optical analytical systems.

Composed of polystyrene polymer cross-linked with 4-8% divinylbenzene (DVB), these particles are chemically inert and can be washed with alcohol, and then vacuum or air dried.

The polymer density is 1.05 g/cm³ and the index of refraction is 1.59 @ 589 nm. They are packaged as aqueous suspensions at 10% solids by weight.

Note: 15 mL ("A" bottles) listed are available for immediate purchase. 100 mL ("B" bottles) and 1000 mL ("C" bottles) are packaged to order, i.e., 7503B and 7503C.

To order Thermo Scientific 7000 Series particle products, call 1-800-232-3342 (USA) or 1-510-979-5000 (International).

Specifications

Composition: Polystyrene crossed linked with divinylbenzene (DVB)
Density: 1.05 g/cm³
Index of Refraction: 1.59 @ 589 nm (25°C)
Additives: Contains trace amount of surfactant

Nominal Diameter	Size Uniformity	Bottle Size	% Solids	Catalog Number
3.2 µm	≤ 45%	15 mL	10%	7503A
6.0 µm	≤ 25%	15 mL	10%	7505A
7.9 µm	≤ 20%	15 mL	10%	7508A
11 µm	≤ 18%	15 mL	10%	7510A
17 µm	≤ 16%	15 mL	10%	7516A
19 µm	≤ 16%	15 mL	10%	7520A
25 µm	≤ 15%	15 mL	10%	7525A
45 µm	≤ 15%	15 mL	10%	7545A
55 µm	≤ 16%	15 mL	10%	7550A
71 µm	≤ 15%	15 mL	10%	7575A
90 µm	≤ 16%	15 mL	10%	7590A
97 µm	≤ 12%	15 mL	10%	7602A
134 µm	≤ 16%	15 mL	10%	7640A
222 µm	≤ 12%	15 mL	10%	7725A

Thermo Scientific Smoke-Check

Smoke Detector Challenge Particles

This complete testing kit checks the transport time of air sampling smoke detectors as required by the United States NFPA Standard 72.

A suspension of polystyrene particles in high purity water is nebulized using a handheld ultrasonic atomizer to mimic natural smoke, thereby eliminating residues caused by traditional oil-based sprays or real smoke.

This makes Smoke-Check especially useful in cleanrooms and other critical environments.

The particle size and concentration have been optimized to consistently trigger the smoke detector.

In addition, the particles will not pass through HEPA filters, ensuring the integrity of the cleanroom environment.

The Smoke Detector Challenge Kit includes a battery powered portable ultrasonic nebulizer, a draft shield and a 10 mL bottle of particles.

Instructions for operating and cleaning the nebulizer are included. Additional bottles of Smoke-Check may be purchased separately.

Specifications

Composition:	Polystyrene
Density:	1.05 g/cm ³
Refractive Index:	1.59 @ 589 nm (25° C)
Additives:	Trace surfactant to inhibit agglomeration

Nominal Diameter Range	Bottle Size	% Solids	Description	Catalog Number
Not available	10 mL	5%	Smoke Detector Challenge Particles	SD-01
Not available	Kit	5%	Smoke Detector Challenge Kit	SD-02

Technical Supplement

Selecting Particle Surface Properties For Diagnostic Applications

Polymer particles are used in diagnostics for lateral flow chromatographic strip tests, latex agglutination assays, suspension array tests and nephelometric assays.

There are wide variations in particle composition, surface properties and size control that can affect the performance of a diagnostic reagent, which is why it is important to gain a better understanding of the particle selection process.

The Importance of Particle Surface Properties

The surface of particles is one of the most important properties for particles used in diagnostic tests and other applications where proteins and other biomolecules are bound to the surface.

Residual surfactants, monomers and microbial contamination can interfere with the successful conjugation to the particles. These contaminants are often the cause of batch-to-batch non-reproducibility of the conjugation reactions, and these variations can interfere with the production process for diagnostic tests.

Careful control of the particle diameter is also important since the surface area changes exponentially with the changing diameter. Variations in surface area can cause apparent changes in sensitivity. Consistency in particle manufacturing and quality control assures that these problems will not occur.

The functional groups available on the surface of the particles control the chemistry of the conjugation process and directly influence sensitivity and stability. Selecting particles with the appropriate surface and quality characteristics is the key to developing stable, reproducible diagnostic tests.

Following is a discussion of how surface properties effect two broad categories of biomolecular conjugation.

Properties Affecting Hydrophobic Adsorption

Particles with sulfate and carboxyl groups are designed for hydrophobic (passive) adsorption.

The particle surface is very hydrophobic, with a low density of negatively charged surface ions to provide charge stabilization. These particles will bind to any molecules that are characteristically hydrophobic, including proteins, peptides and small hydrophobic molecules.

The binding affinity tends to increase as molecular weight increases, and can result in the preferential binding of higher molecular weight proteins in mixtures.

Specific adsorption of substances such as antibodies is easily accomplished by mixing the particles and the protein together at an optimal pH and then separating the unbound protein from the solid phase, usually by centrifugation or cross-flow filtration.

The charge groups on these particles are derived from the initiators used in the synthesis of the particles, resulting in either sulfate or carboxyl ionic groups on the particle surface. The main difference between these two types of hydrophobic particles is their pH stability. Sulfate particles are stable above pH 3, while carboxyl particles are stable above pH 6.

There are other more subtle differences, and these come into play when one or the other particle types give a superior result when antibody is bound to its surface.

Binding, storage and reaction buffer conditions are particularly important parameters that must be optimized. See our MP reagent OPT manual for more information.

Properties Affecting Covalent Coupling

Carboxylate-modified and aldehyde-modified particles are designed for covalent attachment by reaction with amines.

The modified particles are made from sulfate particles by grafting a copolymer containing the desired chemical group onto the surface, producing a thin, relatively hydrophilic polymeric layer. This results in a high density of carboxyl or aldehyde surface groups that can be chemically activated to give a reactive intermediate that will couple with amines on proteins and other biomolecules.

Carboxylate-modified particles differ from the hydrophobic carboxyl particles in that the surface is somewhat porous, more hydrophilic and has a relatively high charge density of 10-125 Å² /carboxyl.

These particles are more stable in the presence of high concentrations of electrolytes (up to 1 M univalent salt).

Unlike the hydrophobic carboxyl particles, the high density and better availability of the carboxyl groups on these particles facilitate reaction with protein amines after activation with carbodiimide reagents.

Alternatively, one can convert to the active esters in a two-step coupling reaction process. See page 10 for our recommended coupling procedure.

Aldehyde-modified particles have aldehyde groups grafted to the surface and can react with protein amines through Schiff base formation. The aldehyde-modified particles do not require chemical activation and thus offer a convenient one-step method of covalent attachment.

Amine-modified particles are prepared from carboxylate-modified particles by converting some of the carboxyl groups to amine groups. The resulting amine modified particles still retain a net negative charge to ensure good charge stability, and can easily be coupled to antibodies and other proteins using a variety of bifunctional linkers.

This conjugation approach offers a different way of attaching molecules to the particle surface.

Particle Manufacturing Quality

With so many particle variables affecting the reagent-making process, it is essential that all phases of particle design and production be tightly controlled in a reproducible environment. This is a strong contribution to reagent batch repeatability.

View our technical notes at www.thermoscientific.com/particletechnology

Meeting Your Particle Technology Needs

Ordering Information

When ordering samples or evaluation packs, please specify product volume and catalog numbers. When re-ordering (“approved”) bulk material, please advise us of specific manufacturing lot numbers and volume. Nominal diameters, surface acid content and binding capacities are listed in the product tables. Exact parameters and other technical information is listed on package inserts and certificates of analysis.

Not all particles are available at all times. For specific product and lot availability, please contact Customer Service at 1-800-232-3342 or by email at info.microparticles@thermofisher.com.

Although we can manufacture up to 300 liter batch sizes, please advise us of your particle usage requirements so that we can confirm sufficient inventory quantities for your application.

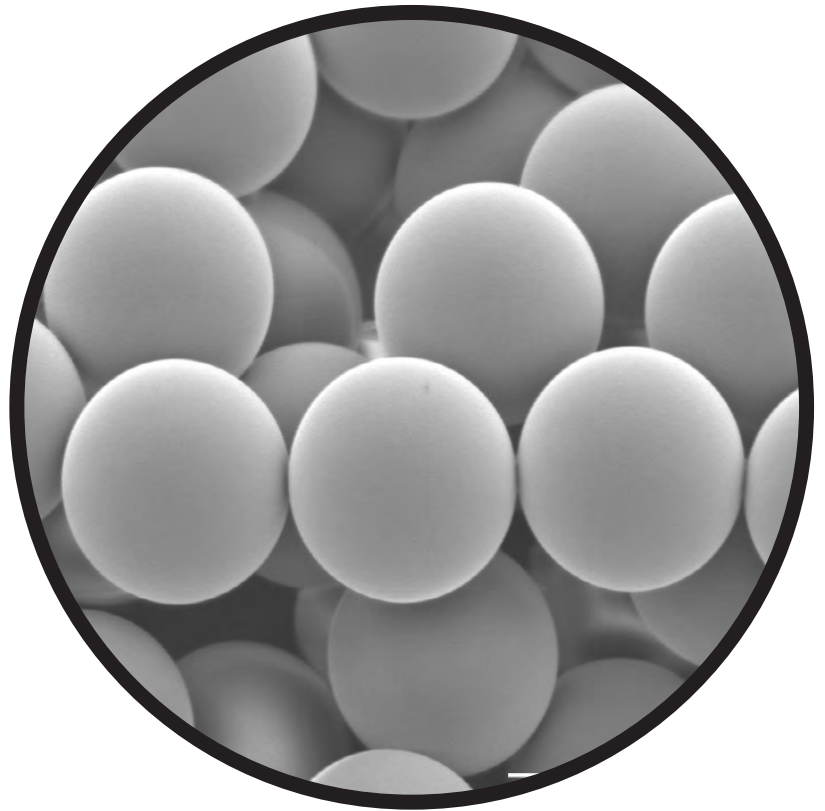
Placing an Order

For faster service when placing an order, please provide the following information to our Customer Service Department:

- Your account number
- Your purchase order number
- Your contact name, phone number, email
- Requested delivery date
- Your “ship-to” address
- Shipping requirements (indicate any special requirements)
- Our part/catalog number (if known)
- Our part description
- Package size/unit of measure
- Quantity of packages
- Our quote number and price (if provided)
- Specification (if any)

Sample Evaluation Packs

Some of our particle products are sold in economical sample evaluation pack form. These packs allow you to buy small samples from our standard inventory of different surfaces, colors, sizes and binding capacities at greatly reduced prices.



Specifications and availability subject to change without notice.

Meeting Your Particle Technology Needs

Product Returns

No returns are accepted without prior authorization. Please contact Customer Service for assistance.

Payment Information

Thermo Fisher Scientific sales terms are net 30 days from the date of invoice for companies that have an established credit account with us.

To establish a credit account, please request and complete our credit application form. If you have any questions, please contact our Customer Service Department.

MSDS

Material Safety Data Sheets are available for all Thermo Scientific products. Please contact our Customer Service Department or visit www.thermoscientific.com/particletechnology if you need more information.

Quality Commitment

Thermo Fisher Scientific provides high quality manufacturing in our ISO 13485 certified facilities. We are FDA registered as an in vitro diagnostic device manufacturer and are cGMP compliant.

Technical Support

Our Technical Service personnel are trained to respond to your needs with specific, expert solutions. Call 1-800-232-3342 (USA) or 1-510-979-5000 (International) with any questions. Our technical notes can also be a resource for projects you are working on. Just visit www.thermoscientific.com/particletechnology for more information.

Customer Service Department

Our Customer Service Department can be reached by telephone, fax or email:

Telephone: 1-800-232-3342 (USA)

1-510-979-5000 (International)

Fax: 1-510-979-5498

Hours: 7 a.m. - 5 p.m. Pacific Standard Time

Email: info.microparticles@thermofisher.com

www.thermoscientific.com/particletechnology





© 2011 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.

CT6000.1_1/11

Clinical Diagnostics
Particle Technology

46360 Fremont Blvd.
Fremont, CA
94538

USA: 1-800-232-3342
International: 1-510-979-5000

www.thermoscientific.com/particletechnology
info.microparticles@thermofisher.com

Thermo
SCIENTIFIC