PolyFacts
Microspheres/Particles
Vol. 6 | No. 2

News | Views | Insights *from*

Polysciences, Inc.
Chemistry beyond the ordinary

You Can Count On Us!

SureCount™ Standards for Validation of Liquid Particle Counters

Particle counting instruments are employed in many research fields and commercial industries. This technology is used to assess the effectiveness of laboratory processes such as water filtration, and to determine particulate levels in environmental water samples. Automated particle counters are used to support industrial contamination control programs and also in the evaluation of finished products such as ultrapure chemicals or pharmaceutical parenterals.

Though the application of particle counting technology is diverse, there is a common need for instrument validation and ongoing QC. Microsphere-based particle count standards may be used to validate liquid counters across their dynamic ranges and to ensure continued capability through the performance of daily QC checks. The use of a reference material permits the standardization of results between runs, instruments and laboratories, and over time.

Polysciences is pleased to announce the availability of **SureCount™ standards** for the validation of particle counters and supporting sample preparation processes. SureCount™ standards are available in **3µm, 5µm, 10µm and 15µm diameters**, and all diameters are traceable to NIST Standard Reference Materials. The standards are supplied as 1 x 10^6 microspheres/ml aqueous suspensions in 10ml volumes.

SureCount™ microspheres complement our existing catalog of standards for analytical instruments, including viability analyzers, particle sizers, flow cytometers and microscopes.

Cat. No.	Description
25379	**SureCount™ Standards, 3µm**
25380	**SureCount™ Standards, 5µm**
25381	**SureCount™ Standards, 10µm**
25382	**SureCount™ Standards, 15µm**

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Binding Us Together!

Flow Cytometry Protein A and Protein G Antibody Binding Beads (Cat. #BLI853 and Cat. #BLI854) are now available! Single population Protein A or Protein G microspheres are suitable for labeling with conjugated antibodies from a range of hosts. These beads may be used as single-population reference standards or in conjunction with an unlabeled population for compensation purposes. Try them today!

Violet is the New Blue!

Not One, but Two New Standards for the Violet Laser

Violet lasers (405nm) have grown increasingly important for multicolor analyses on the flow cytometer. They not only expand the capabilities of the instrument in general, they are important for specific types of analyses, e.g. cell cycle analyses featuring violet-excitable dyes.

As with other components of the cytometer, it is important to use appropriate tools to ensure that the laser and its corresponding detectors, and the system in general, are performing satisfactorily. To this end, Polysciences is pleased to announce the introduction of two new dedicated standards for the violet laser, the **Violet Laser Reference Standard**, and **Simply Cellular® anti-Mouse for Violet Laser**.

The **Violet Laser Reference Standard** may be used for **daily QC and set-up** of fluorescence channels off of the 405nm laser, and also as a **relative intensity standard** for tests and assays featuring Alexa Fluor® 405, Pacific Blue™ or other violet-excitable fluorophores. As an internally-dyed bead, it exhibits exceptional stability for tracking instrument performance or study results.

Common polymer compositions (polystyrene, etc.) typically possess a significant absorbance band in the UV / Violet region. This can lead to seemingly higher detection thresholds and complicate bead-based compensation when using 405nm excitation. The **Simply Cellular® anti-Mouse Violet Laser** standard features microspheres comprised of a proprietary matrix that exhibits **low autofluorescence with violet excitation**. Beads are suitable for labeling with mouse mAbs conjugated with violet fluorochromes, and for use as a **compensation or general reference standard** for detectors off of the violet laser. The Simply Cellular® anti-Mouse for Violet Laser standard is supplied as 2 populations: 1 blank and 1 high binding anti-Mouse IgG (Fc-specific) population.

Figure 1: *Autofluorescence With 405nm Excitation*

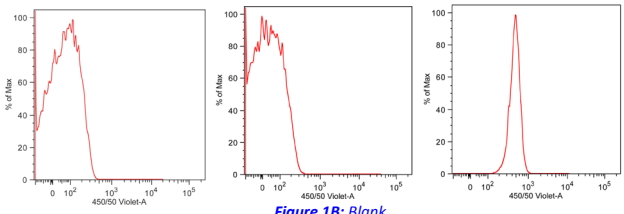

Figure 1A: *Unstained Cells*

Figure 1B: *Blank Simply Cellular® Violet Microspheres*

Figure 1C: *Blank Polymer Microspheres*

Figure 1A, 1B, 1C: *With 405nm excitation, unlabeled Simply Cellular® for Violet Laser beads demonstrate autofluorescence resembling that of unstained cells. Unlabeled polymer beads demonstrate greater autofluorescence with violet excitation.*

Cat. No.	Description
BLI915	**Violet Laser Reference Standard**
BLI835	**Simply Cellular® anti-Mouse for Violet Laser**

Tired of Being Suspended?

Dry silica microspheres are now available! Oh, and Larger Diameters, too!

We at Polysciences are pleased to announce our **newly-expanded catalog of silica products!** Both **larger diameter** offerings (>5µm) and **dry preparations** of plain silica microspheres join our existing streptavidin, amine, carboxyl and traditional unmodified silica spheres. New large silica products include 6, 7 and 8µm diameters, and dry silica microspheres are available in 9 discrete diameters from 0.3µm – 6.0µm to suit a variety of applications.

Exhibiting low autofluorescence across the visible spectrum, silica microspheres offer significant advantages to fluorescence-based technologies. They are naturally hydrophilic, and may be modified using commonly available silanes to support specific surface chemistries or tailor physical properties. They are able to withstand high heat and solvent treatment, as well as rigorous processing. Dry microspheres offer an added convenience to our customers who may be intermixing them with unique carrier fluids or using them in a dry state.

Big, small, coated, functionalized, plain, dry or suspended silica – we have them all!

U.S. & Canada: (800) 523-2575 / (215) 343-6484 **Fax:** (800) 343-3291 / (215) 343-0214 **email:** info@polysciences.com

Particle Perplexities

Questions & Answers Pertaining to Polysciences' Microspheres / Particles

Q: I want to coat microspheres with antibody. How can I determine the capacity of polystyrene beads for protein?

A: The amount of protein that may be coated on a surface will largely depend upon the size of the protein as well as the surface area of the bead. Binding properties will also be a function of the affinity of the specific molecule for the surface of the bead. **Cantarero, et. al.** studied the capacity of polystyrene surfaces for proteins such as albumins and bovine immunoglobulins, and from this work, an equation describing a microsphere's potential coating capacity was derived:

$$S = \frac{6}{\rho_S d} \bullet C$$

where: S = amount of representative protein required to achieve surface saturation (mg protein / g microspheres)
ρ_S = density of solid sphere (g/cm^3)*
d = mean diameter (µm)*
C = capacity of microsphere surface for a given protein (mg protein/m^2 of sphere surface)

Notes: a. C ~3 mg/m^2 for BSA [MW 65kD],
C ~2.5 mg/m^2 for bovine IgG [MW 150kd].[1]

Using a mean diameter of 1.0µm:

For BSA: C ~3 mg/m^2, so:
$S = (6 / \rho_S d)(C)$
$= (6 / [1.05\ g/m^3 \bullet 1.0\mu m])(3\ mg/m^2)$
~ 18mg of BSA to saturate 1 gram of 1µm polystyrene-based microspheres.

For BIgG: C ~2.5 mg/m^2, so:
$S = (6 / \rho_S d)(C)$
$= (6 / [1.05\ g/m^3 \bullet 1.0\mu m])(2.5\ mg/m^2)$
~ 15mg of BIgG to saturate 1 gram of 1µm polystyrene-based microspheres.

Resulting values are considered to equal a protein monolayer. When developing a coating protocol, we suggest using an amount of protein that is equivalent to 1 – 10X this value as a starting point. Performance of a small scale protein titration should allow further optimization of protein concentration.

The protocol that is provided with our **PolyLink Protein Coupling Kit (Cat. 24350)** also provides a good starting point, and may similarly be optimized for use with different proteins and bead diameters.

Cantarero LA, JE Butler, JW Osborne. (1980) The adsorptive characteristics of proteins for polystyrene and their significance in solid-phase immunoassays. Anal Biochem; 105:375-382.

Cat. No.	Description
24350	**PolyLink Protein Coupling Kit**

Q: I want to surface-label my 0.5µm functionalized polystyrene beads with a compound that is insoluble in water. The compound is currently in DMSO, but I'm afraid that the beads might be damaged if I try coupling it to the beads in solvent. Do you know of any organic solvents that are compatible with polystyrene spheres or can you advise how I might be able to identify an appropriate solvent for my labeling reaction?

A: As a first approach, rather than suspending the polystyrene beads in solvent, a common strategy is to add the compound (solvent) to the beads (aqueous). This effectively dilutes the solvent, and should protect the beads from ill effects (significant swelling or dissolution).

If the beads really must remain in an organic system, there are fortunately a few organic solvents that are chemically compatible with polystyrene including methanol, ethanol, and some linear saturated hydrocarbons, etc. We actually call these compatible liquids "non-solvents" because polystyrene beads are unlikely to swell or dissolve when suspended in such environments. "Solvents" on the other hand will attack the polystyrene matrix, especially if the microspheres are not crosslinked with divinylbenzene (DVB). Our submicron polystyrene beads are not crosslinked with DVB, so investigators should take care to avoid solvent usage when working with smaller spheres unless bead destruction is the intended goal (hey, you never know).

A good resource for determining bead/solvent compatibility is the Polymer Handbook (Brandrup et. al.). A good rule of thumb for solubility of a polymer in a solvent is that the solubility parameters are within 1 unit of each other or less (for swelling or dissolution). Of course, now we're talking chemistry, so there is some room for negotiation. But, to keep the beads happy, you probably should try to find a suspending liquid whose solubility parameter is not a numerical neighbor to that of polystyrene.

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