PolyFacts

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Microspheres/Particles

News | Views | Insights *from* Polysciences, Inc. Chemistry beyond the ordinary

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Good Things Come in Small Packages...

Introducing 1µm ProMag™

We are delighted to announce the addition of our new 1µm ProMag™ to the Polysciences' catalog. Like its 3µm counterpart, it boasts stringent size uniformity, fast and uniform separations, high coating capacity and low nonspecific binding of proteins.

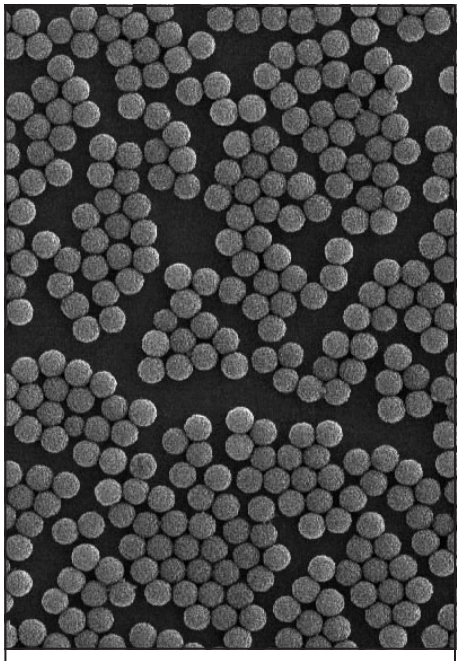
The surfactant-free carboxyl version is ideal for covalent binding of biomolecules, such as antibodies, peptides and oligonucleotides. Streptavidin ProMag™ may be easily coated with biotinylated ligand or used to capture biotinylated targets, such as PCR amplicons or labeled cells.

The smaller (1µm) size offers far greater surface area per unit weight, which can present a considerable advantage for the purification of biomolecules or capture of low concentration targets. Smaller-diameter spheres additionally remain suspended for longer periods of time, simplifying assay incubation steps.

All of this, plus the convenience and ease of magnetic separation. It doesn't get much better than that.

ProMag™ 1µm - little bead, big love.

Cat. No.	Description
25029	**ProMag Series 1 • Carboxyl**
25031	**ProMag Series 1 • Streptavidin**



ProMag™

We're Going Buff!

Introducing our New Microsphere Solutions and Buffers

Going buff, you say?! We're not talking about going around in the buff, painting our offices buff, buffing our cars, isolating buffy coats or even becoming movie buffs (though we are pretty enthused about the current box office line-up). Never mind all of that – we're talking about our new line of microsphere solutions and buffers!

We are pleased to present our new range of solutions and buffers for plain, dyed or functionalized polymer microspheres. These ready-to-use aqueous buffers take the guesswork out of buffer selection and are available in different formulations to accommodate a range of chemistries and biomolecules throughout the microsphere life cycle.

Figure 1: *Polysciences Bead Solution*

Figure 2: *Bead Coupling and Storage Buffers*

The Polysciences Bead Solution is a general-purpose solution for the dilution and/or storage of your uncoated, plain, dyed or functionalized polymer microspheres. An antimicrobial agent deters microbial contamination and stabilizers promote suspension dispersity. Our Coupling Buffers, with values ranging from pH 4.5 to pH 9.0, are suitable for a variety of conjugation chemistries and biomolecules. Rounding things out are our Storage Buffers in pH 7.4 or pH 8.5, which also contain an antimicrobial agent and stabilizer for lasting shelf life. The Coupling Buffers and Storage Buffers are available in 250ml, 500ml, 1000ml and 2000ml volumes and the Bead Solution in volumes of 500ml, 1000ml and 2000ml.

Cat. No.	Description
24973	**Polysciences Bead Solution**
24976	**Polysciences Bead Coupling Buffer, pH 4.5**
24977	**Polysciences Bead Coupling Buffer, pH 6.0**
24974	**Polysciences Bead Coupling Buffer, pH 7.4**
24978	**Polysciences Bead Coupling Buffer, pH 9.0**
24979	**Polysciences Bead Storage Buffer, pH 7.4**
24975	**Polysciences Bead Storage Buffer, pH 8.5**

So, no more agonizing over the perfect formulation, performing head-scratching calculations or working to exhaustion measuring and mixing reagents. No, that's for us to do. Just take it easy (i.e. buy a buffer) and enjoy all of the extra free time!

Oh, and if the solution or buffer you need is not listed above, please visit the Buffers page in the *BioSciences* section of our website at **www.polysciences.com** for a listing of additional liquid concentrates, powdered blends and buffer components.

You Light Up My Life!

Fluorescent Microspheres for (Pretty Much) Every Application

Fluorescence remains one of the most used technologies in life science research and diagnostics. Fluorescent microspheres are used to label biologic samples, as solid supports in diagnostic assays and to support the many instruments used for these analyses.

It's a tall order, but we can help! The Polysciences' catalog includes fluorescent beads from 50nm - 90µm for a range of diagnostic, imaging and calibration purposes. Our Fluoresbrite® microspheres are general-purpose beads that are well suited for applications in microscopy, where an intense and stable signal is required. Microspheres are available in plain, carboxyl and amine versions to support a range of conjugation strategies if coating is required.

Our collection of dedicated flow cytometry standards includes many products that are labeled with traditional fluorochromes at levels that approximate stained cell samples. Beads may be used to set up and QC flow cytometers and as intra-assay standards.

Basically, we have a lot of options to support a lot of applications, and if you're unsure about what to use or need something special, just give us a call!

We love to talk about beads!

Particle Perplexities

Questions & Answers Pertaining to Polysciences' Microspheres / Particles

Q: I have a peptide that I'd like to attach to polymer spheres, but I don't know where to start. What are your suggestions?

A: From a coating standpoint, the ideal scenario would feature a synthetic peptide with a tag that could be used for end-point attachment, such as a terminal biotin or polycysteine tag. Biotinylated peptides could be easily immobilized to streptavidin-coated beads; for example, some protocols are as simple as incubating the two for thirty minutes at room temperature in a standard buffer. As for a polycysteine tag, there are a number of commercially-available cross-linking reagents that are amine- and sulfhydryl-reactive, which would allow the targeted binding of Cys-SH groups to amine-functionalized microspheres. Use of synthetic peptides also presents the opportunity to incorporate a spacing chain or sequence into the tag.

If you elect to covalently couple the peptide (rather than use streptavidin/biotin), I would suggest a two-step procedure. This would involve modification of the bead with crosslinker, then a wash to remove excess crosslinker, followed by reaction with peptide. Removal of the excess reagent safeguards against peptide crosslinking.

We have coupled synthetic peptides to beads using standard aqueous chemistries. If the peptide needs to be solubilized in an organic solvent, it may be possible to do so, then add it to the aqueous bead suspension. In this scenario, the diluted organic is usually not harmful to the beads.

Q: I recently bought a batch of fluorescent microspheres to make a slide with fixed beads for imaging calibration purposes. I was using a UV adhesive and a strange thing happened. When I resuspended the beads in the adhesive, after about thirty minutes, they lost their fluorescence. What went wrong?

A: I suspect that one of the components of the adhesive is acting as a solvent; for example, solvent may be included in the formulation to retain flow properties before curing. Fluoresbrite® microspheres are internally dyed with water-insoluble dyes. Organic solvents will swell the polymer matrix and allow release of the fluorophore, thus the "disappearing microsphere" phenomenon. However, a water-soluble polymeric mounting medium, such as Mowiol® 4-88 (Cat. #17951, TDS 777), will be compatible with fluorescent polymer microspheres.

Another option would be to purchase prepared slides, as featured in the Confocal Microscopy, Multifluorescent Adjustment & Calibration Kit (Cat. #24016). The kit includes:

- One coverglass mirror mounted with Mowiol®
- Two slides with 200nm multi-fluorescent particles in Mowiol®
- Two slides with 500nm multi-fluorescent particles in Mowiol®
- One slide with 1µm multifluorescent particles in Mowiol®

TDS #520 provides additional information regarding the multifluorescent beads, which are internally dyed with three different fluorescent dyes and have excitation maxima of 377, 517 and 588nm, and emission maxima of 479, 546 and 612nm. As the slides were made using an aqueous mounting medium, beads do not swell and release the entrapped fluorescent dyes.

Cat. No.	Description
17951	**Mowiol® 4-88**
24016	**Confocal Microscopy, Multifluorescent Adjustment & Calibration Kit**

Q: Though I've been working with polymer microspheres for some time, it seems that the bead concentration of my final coated product (after antibody conjugation and wash steps) varies significantly from batch to batch. What can I do to improve yield, and what is the best way to determine the final bead concentration?

A: Though we expect some bead loss with standard handling and processing, excessive loss may point to hydrophobicity-associated bead loss or inefficient separations in general. Indicators of severe hydrophobicity may include beads floating on the surface, smearing on the tube wall during centrifugation steps or clinging to pipette tips or tubes. This can worsen with successive washes, as residual surfactant diminishes. This can be remedied by adjusting the centrifugation protocol (greater force/time) or conducting some of the washes in surfactant-containing buffer. However, as surfactant is expected to compete with the ligand for the surface of the microsphere, a balance needs to be achieved between handling characteristics and binding efficiency.

If you don't feel that hydrophobicity is the culprit, your centrifugation protocol may need to be adjusted. Provided that spheres are of sufficient size, viewing a sample of supernatant using a standard microscope will tell you if beads are not being fully pelleted. At 400X magnification, individual 1µm+ spheres should be visible.

Determining the bead concentration can be accomplished using a variety of methods. We often use a gravimetric method, though this may not be practical for assessing small volumes due to the amount of material consumed. Particle counters are also very useful, e.g. automated counting using a Coulter counter or manual counting using a hemacytometer.

We Smell a Rat...Again

Introducing Our Newest anti-Rat Standards for Flow

We're not actually anti-rat, not at all. Rather, we are delighted to introduce our latest binding standards for rat primary antibodies. Our new Simply Cellular® anti-Rat product is a single population of microspheres coated with anti-Rat IgG (Fc specific) antibody for labeling with fluorescent monoclonal rat antibodies. It may be used in conjunction with a suitable Quantum™ MESF kit for determination of the antibody's fluorophore labeling density (effective fluorophore / protein [F/P] ratio) or used to QC the fluorescence intensity of different antibody lots or clones.

The new Simply Cellular® anti-Rat Compensation Standard complements our other products for compensation in multicolor flow cytometry. It includes a mixed population of low- and high-binding beads that users label with antibody conjugates to establish test-specific compensation settings.

But we're not just about rats...check out our complete line of products for use with mouse, rat and human primary antibodies. To find out more, visit us at www.polysciences.com.

Don't forget QuickCal®, which is provided FREE with our Quantum™ flow cytometry products for quantitating fluorescence intensity.

Cat. No.	Description
BLI551	**Simply Cellular® anti-Rat Compensation Standard**
BLI813	**Simply Cellular® anti-Rat IgG**
BLI817	**Quantum™ Simply Cellular® anti-Rat IgG**
BLI550	**Simply Cellular® anti-Mouse Compensation Standard**
BLI810	**Simply Cellular® anti-Mouse IgG**
BLI815	**Quantum™ Simply Cellular® anti-Mouse IgG**
BLI552	**Simply Cellular® anti-Human Compensation Standard**
BLI812	**Simply Cellular® anti-Human IgG**
BLI816	**Quantum™ Simply Cellular® anti-Human IgG**

Have the (Trypan) Blues?

Try ViaCheck™ Standards for Cell Viability Analyzers

If wall-to-wall cell cultures have been getting you down, you may be ready to make the leap to an automated viability analyzer. And why shouldn't you? Instrumental methods for cell count and viability provide significant advantages over manual determinations, offering high accuracy, precision and throughput.

However, as most analytical instruments do not include an internal standard to verify results, it's important to use external standards to confirm capabilities and ensure optimal performance on an ongoing basis. Our ViaCheck™ Viability Control and Concentration Standards respond to this need by mimicking the optical characteristics of live and dead cells in the trypan blue dye exclusion method. Non-biological surrogates remove the need for significant sample preparation, and offer exceptional stability and reproducibility. ViaCheck™ microsphere standards are pre-stained, and need only be loaded into the analyzer for confirmation of live/dead ratios and counts. They are available in a range of common concentrations and viability ratios.

ViaCheck™ Viability and Concentration Standards: chasing the blues away one analyzer at a time.

Cat. No.	Description
24622	**ViaCheck™ Viability Control 0%**
24623	**ViaCheck™ Viability Control 50%**
24624	**ViaCheck™ Viability Control 75%**
24625	**ViaCheck™ Viability Control 90%**
24626	**ViaCheck™ Viability Control 100%**
24627	**ViaCheck™ Concentration Control (1 x 10^6)**
24628	**ViaCheck™ Concentration Control (4 x 10^6)**
24629	**ViaCheck™ Concentration Control (8 x 10^6)**

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