TechNote 101

ProActive® Microspheres

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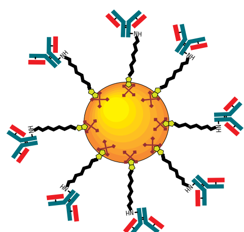
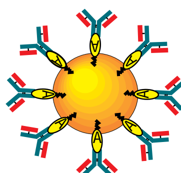
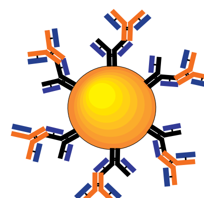
BEADS ABOVE THE REST™



Streptavidin-Coated Microspheres



Protein A-Coated Microspheres



Secondary Antibody-Coated Microspheres

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I. BACKGROUND

Microspheres pre-conjugated to various types of generic binding proteins and secondary antibodies are rapidly becoming the solid phase support of choice in many areas, including immunological applications, nucleic acid work, and cell separation and visualization. These offer several advantages in terms of ligand attachment over traditional plain or surface-functionalized microspheres.

1. Virtually any biological ligand can be biotinylated through a one-step chemical reaction. These will then bind directly to our streptavidin-coated microspheres with a bond strength approaching that of a covalent bond (Ka=10^{15}/M). In addition, because the biotinylated ligand is set off from the surface of the microspheres, the steric hindrance that can cause a problem when coupling to functionalized microspheres is reduced, especially for larger ligands.
2. When binding biological ligands to solid supports, such as microspheres, the orientation by which they bind is often crucial. For example, antibodies should be bound at their Fc (rather than Fab) region to maintain optimum biological activity. All of our protein-coated microspheres are formulated to optimize this orientation.
3. Our streptavidin-coated microspheres are assayed in terms of their ability to bind biotinylated alkaline phosphatase, our Protein A-coated microspheres are assayed for human IgG binding capacity, and our anti-Mouse secondary antibody-coated microspheres are assayed for their capacity to bind mouse IgG. These values allow for easy optimization of reagent requirements, without the added time and expense of a completely empiricial determination of ligand concentration.
4. We use a wide variety of microsphere types for protein coating, including polystyrene (sub-micron to 10µm), silica (sub-micron to 5µm), and superparamagnetic polystyrene (nominally 1µm). This allows for a variety of approaches when separating from an aqueous phase, a process that can be difficult and time-consuming with other types of solid supports.
5. These protein-coated microspheres are available in dyed form, with colors like blue, red, or fluorescent. This can be of great advantage when visualization is required, as is the case in many immunological and cytological applications.

II. STREPTAVIDIN- AND BIOTIN-COATED MICROSPHERES

The avidin/biotin interaction is one of the strongest non-covalent bonds (K_a=10^{15}/M vs. 10^7-10^{11}/M for antibody-antigen interactions).[1] This complementarity, combined with the small size of biotin (MW=244.3), yields an ideal system for affinity binding, with numerous applications in areas such as immunology and cell / molecular biology. Although our microspheres are designed as a solid support with maximal binding and minimal nonspecific

binding, this avidin / biotin system does have its limitations. Avidin has carbohydrate moieties in its molecular structure, which can result in nonspecific interactions with many proteins. Also, avidin's pI of approximately 10 (a net positive charge at neutral pH), can result in nonspecific interactions when working with negatively charged ligands, such as nucleic acids.[2]

One solution to this is to use streptavidin, a tetrameric protein with four biotin binding sites that is similar to avidin in its molecular structure, yet lacks the carbohydrates that can result in nonspecific interactions. Streptavidin's pI of approximately 5 (a net negative charge at neutral pH) avoids nonspecific charge interactions with negatively charged ligands1.

Our streptavidin-coated microspheres have been fully characterized in terms of their ability to bind biotinylated alkaline phosphatase (B-ALP, MW~160kD), and therefore will require minimal optimization when determining the correct concentration of ligand to be bound. Similarly, our biotin-coated microspheres have been fully characterized in terms of their ability to bind free avidin.

Both streptavidin and SuperAvidin™ are biotin-binding proteins. At this time, our standard product is streptavidin-coated microspheres. SuperAvidin™-coated microspheres are research products. SuperAvidin™ is a proprietary molecule that has been found to outperform streptavidin in certain applications, in terms of higher binding of biotinylated ligands and lower nonspecific binding.

A. Physical Parameters

Microsphere Types:

Polystyrene...................... 0.02-10.00µm, plain or dyed in a variety of colors, including fluorescent.

Superparamagnetic Polystyrene...................... Polydisperse or monodisperse polystyrene/ magnetite with nominal mean diameter of ~1µm.

Two types: Classical, with magnetite exposed at surface, and Encapsulated, with outer polymer shell. Magnetite percentage ranges from 12-66% by weight (density ranges from 1.16-2.24 g/cm^3).

Silica.............................. 0.15-5.0µm (density=1.96 g/cm^3)

Concentration: 10mg microspheres/mL (1% solids w/v)

Storage Buffer: 100 mM Borate, pH 8.5 + 0.01% BSA + 0.05% Tween® 20 + 10 mM EDTA + ≤0.1% NaN_3 (unless otherwise specified).

Binding Capacity: Supplied on the Certificate of Analysis for each lot.

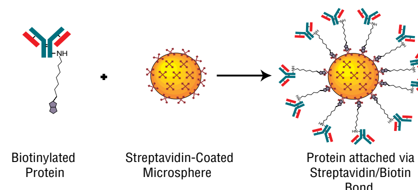
Stability and Storage: Polystyrene and superparamagnetic polystyrene are stable at 2-8˚C for 2 years from date of manufacture, (as determined by accelerated stability testing at 22˚C).

B. Sample Procedures

1. Preparation of Streptavidin-Coated Microspheres

Allow microsphere suspension to come to room temperature, then vortex for approximately 20 seconds before use. A preliminary wash is necessary with most applications, to remove various additives including EDTA, antimicrobials, and surfactants. Several washing methods are possible, and a detailed description of these can be found in our TechNote 203, *Washing Microspheres*.

2. Attachment of Biotinylated IgG / Elution of Purified Antigen

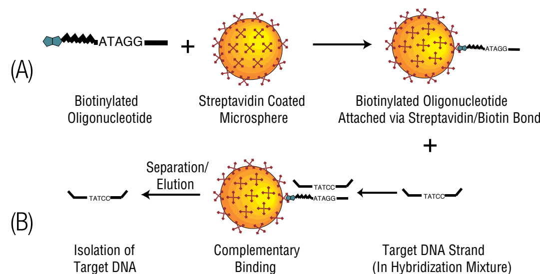


Reagents:
1. Streptavidin-coated microspheres (supplied at 1% solids)
2. Wash buffer (0.1 M PBS, pH 7.4)
3. Elution buffer (0.1 M glycine-HCl, pH 2.5)

Procedure:
1. Wash an aliquot of particles (1-3 times) with a 10X volume of wash buffer.
2. Resuspend the final pellet in wash buffer to a concentration of 0.05% solids (0.5 mg/mL).
3. To this solution, add your biotinylated IgG that has been dissolved in the same buffer. The protein concentration will have to be optimized, but can be based on the binding capacity of the microspheres, as reported on the Certificate of Analysis for each lot.
4. Incubate at room temperature (22˚C) for 30 minutes with gentle mixing.
5. Wash the particles 3 times with another 10X volume of wash buffer.
6. Resuspend antibody-coated beads in 0.1 M PBS, pH 7.4, to desired storage concentration (often 0.5 mg/mL).
7. If using these microspheres for affinity separation of a particular antigen from a heterogeneous mixture, the bound antigen can be eluted and purified by the suspending microsphere/antibody/antigen conjugate in elution buffer.

3. Attachment of Biotinylated Oligonucleotide

A common application of streptavidin-coated microspheres in molecular biology is to separate nucleotides of interest from solution. By attaching a biotinylated oligonucleotide (A), one has a probe that is easy to manipulate and can be used for a number of applications, such as that shown in (B).[3,4,5,6]



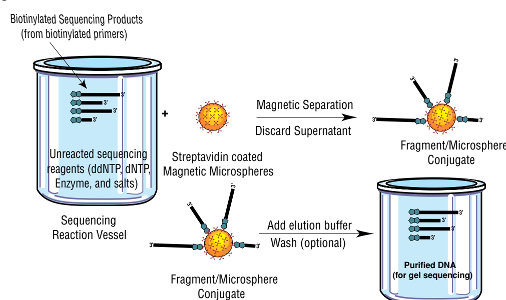
Reagents:
1. Streptavidin-coated microspheres (supplied at 1% solids)
2. Biotinylated oligonucleotide probe
3. 0.15 N NaOH
4. TTL Buffer: 100 mM Tris-HCl; pH 8.0, 0.1% Tween® 20; and 1 M LiCL
5. TT Buffer: 250 mM Tris-HCl; pH 8.0, and 0.1% Tween® 20
6. TTE Buffer: 250 mM Tris-HCl; pH 8.0, 0.1% Tween® 20; and 20 mM Na_2 EDTA, pH 8.0
7. Hybridization Mixture: 2 M NaClO4, 0.4% sodium dodecyl sulfate [SDS]; 20 mM $MgSO_4$; and 10% Polyethylene Glycol (8000 molecular wt.)

Procedure:
1. Transfer 1mg (0.1mL) streptavidin-coated microspheres to a 0.5mL centrifuge tube.
2. Separate, decant, and rinse in 200µL of TTL buffer.
3. Separate, decant, and resuspend in 20µL of TTL buffer.
4. Add biotinylated probe in amount approximating (concentration must be optimized) reported biotin capacity, bringing microspheres to a final volume of 25µL.
5. Incubate for 15 minutes at room temperature (18-25˚C) in TTL buffer with gentle mixing.
6. Separate probe / microsphere conjugates, decant, and wash pellet in 0.15 N NaOH (to remove any nonspecifically bound probe).
7. Rinse 2 times in TT buffer, with appropriate separation steps.
8. Resuspend in TTE buffer, incubate at 80˚ for 10 minutes, and decant to remove any unstable biotin / streptavidin couplings.
9. Resuspend in 100µL of hybridization mixture. Prepared as above, these probe / microsphere conjugates have been stored successfully at 2-8˚C for up to 4 months.

4. Purification of DNA Sequencing Reactions

A fundamental aspect of modern molecular biology is DNA sequence analysis. In order for the DNA to be accurately sequenced, and the background noise reduced, it is necessary to first remove impurities that are remnants of the sequencing reaction (enzyme, salts, unreacted dye terminators, etc.). The following procedure outlines how streptavidin-coated magnetic microspheres can simplify this process.

Standard protocols exist for DNA sequencing.[4,8] Automated sequencing instrumentation is offered by Perkin-Elmer (ABI PRISM®) and Amersham Life Sciences (Thermo Sequenase dye terminator cycle sequencing kit), among others. Therefore, this protocol is specific to using streptavidin-coated superparamagnetic microspheres to purify and isolate DNA for gel sequencing. The reaction is as follows:



Reagents:
1. Streptavidin-coated superparamagnetic microspheres (supplied at 1% solids)
2. Binding Buffer: 1X TES (10 mM Tris-HCl, 1 mM EDTA, 1 M NaCl, pH 8.2) and 0.2% Tween® 20
3. Wash Buffer: 1X TES buffer
4. Elution Buffer: 10 mM EDTA, 95% formamide, 0.05% bromophenol blue (or other DNA stain)

Procedure:
1. Add 20µL of microspheres and 20µL of binding buffer to each reaction vessel (per 50µL volume). *Note:* These volume ratios are a baseline that might need to be optimized for your particular sequencing reaction.
2. Incubate for 15 minutes with gentle mixing.
3. Wash 2 times (by magnetic separation) with 10µL of wash buffer, and 1 time with 10µL of H2O.
4. Elute DNA by resuspending in 6.5µL of elution buffer at 90˚C for 5 minutes, with mixing.

III. PROTEIN A-COATED MICROSPHERES

Protein A is a 42 kD polypeptide that is a normal constituent of the cell wall of *S. Aureus*. It was discovered in early work with this bacterium when researchers noticed that one fraction, isolated during purification of the individual components of the cell wall, contained a protein that would bind to rabbit and human antibodies. Surprisingly, this protein bound to antibodies isolated not only from animals that had been immunized with Protein A, but also from animals that had never been exposed to this antigen.

Although Protein A has four antibody binding sites, only two of these can be used at one time. It is known that there are at least two Protein A binding sites on any antibody, and that these are located in the Fc region of the antibody. Because Fc-directed binding is desired in order to maximize the antibody's biological activity, Protein A pre-conjugated to a solid support, such as our microspheres, has become an important reagent in many immunochemical applications.

Protein A-coated microspheres have a couple of advantages when compared to conventional surface-functionalized microspheres. Antibodies are bound to Protein A beads in a simple one-step process, and the antibody can be eluted if necessary. Traditionally, attaching antibodies to polystyrene microspheres has been done by passive adsorption or covalent coupling. Passive adsorption is the easier of the two, but the possibility of reversibility and undesirable antibody orientation exists. Covalent coupling is irreversible and, with the proper chemistry, allows for controlled orientation of antibody attachment. The disadvantages of any covalent coupling protocol are the time and costly reagents involved in optimization.

Protein A-conjugated microspheres are, for many applications, the answer to these problems. Antibody coupling is Fc-directed (to maximize biological activity), done in one step (simply by mixing the reagents in their proper concentrations), and the bond formed is very strong ($K_a=10^9$)[7], yet reversible at low pH. Antibody can be irreversibly attached by covalently cross-linking the antibody to the Protein A.

Protein A is a very sturdy molecule. Research has been done in which Protein A is subjected to very harsh conditions (6M Guanidine HCl, 4 M Urea, 4 M thiocyanate, or pH 2.5), and in each case, the Protein A was renatured to full binding capacity when returned to normal physiological conditions. While these features make Protein A useful in a wide variety of applications, it does have its limitations. Protein A's affinity for various polyclonal antibodies is species-specific (Table 1).

Table 1: Protein A Affinities for Polyclonal Antibodies from Various Species

Species	Affinity for Protein A
Rabbit, Guinea Pig	++++
Human, Pig	+++
Horse, Cow, Mouse	++
Sheep, Rat	+/-
Hamster	+
Goat, Chicken	-

Also, when using monoclonal antibodies, its affinity for IgGs will vary with the different sub-classes within a species (Table 2).[9]

Table 2: Protein A Affinities for Various Monoclonal Antibodies

Antibody	Affinity for Protein A
Human IgG, IgG_2, IgG_4	++++
Human IgG_3	-
Rat IgG_1, IgG_{2a}, IgG_{2b}	-
Rat IgG_{2c}	+
Mouse IgG_1	+
Mouse IgG_{2a}	++++
Mouse IgG_{2b}	+++

In practice, sera from humans, donkeys, rabbits, dogs, pigs, and guinea pigs can be used without worry for all tests that rely on Protein A. Most immunochemical assays will not be affected by using polyclonal antibodies from mice, cows, or horses. However, depending on the type of assay, antibodies from sheep, goats, rats, or chickens will often need a secondary antibody layer to ensure quantitative binding.

As Table 2 shows, subclasses that are more difficult to use include human IgG_3, mouse IgG_1, and all rat sub-classes, except possibly IgG_{2c}. In all these cases, Protein A may need to be supplemented with a second, bridging antibody layer, or be substituted with another secondary antibody.

A. Physical Parameters

Microsphere Types:

Polystyrene...................... 0.02-10.00µm, plain or dyed in a variety of colors, including fluorescent.

Superparamagnetic Polystyrene...................... Polydisperse or monodisperse polystyrene/magnetite with nominal mean diameter of ~1µm.

Two types: Classical, with magnetite exposed at surface, and Encapsulated, with outer polymer shell. Magnetite percentage ranges from 12-66% by weight (density ranges from 1.16-2.24 g/cm³).

Silica............................... 0.15-5.0µm (density=1.96 g/cm³)

Concentration: 10mg microspheres/mL (1% solids w/v)

Storage Buffer: 100 mM Borate, pH 8.5 + 0.01% BSA + 0.05% Tween® 20 + 10 mM EDTA + 0.1% NaN_3 (unless otherwise specified).

Binding Capacity: Supplied on the Certificate of Analysis for each lot.

Stability and Storage: Polystyrene and superparamagnetic polystyrene are stable at 2-8˚C for 2 years from date of manufacture, (as determined by accelerated stability testing at 22˚C).

B. General Guidelines

Because Protein A's affinity for various antibodies varies (see Tables 1 and 2), some optimization of ligand binding will be required. Some of the parameters that will play a role in optimal binding are as follows:

1. pH: Typically, IgG's bind optimally near pH 8.
2. Salt concentration: Typically that of normal saline is sufficient. However, with many monoclonal antibodies, it is often beneficial to work with salt concentrations greater than 1 M[10].
3. Buffers: Typically, low ionic strength buffers of borate, HEPES, PBS, TRIS, TBS, or sodium carbonate work well.
4. Cations: The presence or absence of divalent cations, such as Mg^{2+} or Ca^{2+}, will often be a factor in binding. Some proteins will only bind to Protein A in the presence of divalent cations, while for others this hinders binding. Therefore, use of a chelating agent, such as EDTA, may affect binding.
5. Temperature: Normally, binding reactions can be carried out at room temperature, but some monoclonal antibodies show enhanced binding at 4˚C.
6. Concentrations: A good starting point to achieve maximum binding of human IgG is at a concentration of 130µg/mL of buffer. Further, microspheres at a concentration of ~10mg/mL often yield optimal binding.

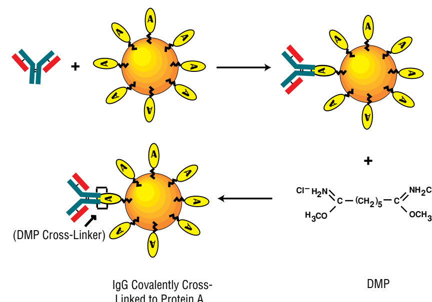
C. Sample Procedures

1. Preparation of ProActive® Protein A-Coated Microspheres

Allow microsphere suspension to come to room temperature, then vortex for approximately 20 seconds before use. A preliminary wash is necessary with most applications to remove various additives, including EDTA, antimicrobials, and surfactants. Several methods are available to wash these, and a detailed description of these can be found in our TechNote 203, *Washing Microspheres*. *Note:* This wash should be performed at pH 4, as this is the pH at which any impurities bound to Protein A will be eluted.

2. Fc-directed Attachment (and Elution) of IgG to Protein A-Coated Microspheres (A) / Covalent Cross-Linking Procedure (B)

Protein A interacts with IgG in such a way that the bond formed is reversible at low pH (2.5). This can be advantageous in many applications, although it is undesirable when forming microsphere / antibody reagents as are used in many immunoassays (a requirement of these reagent conjugates is that they be permanent). The following procedure first details a protocol for attaching an IgG protein to Protein A-coated microspheres, and then uses DMP (dimethyl pimelimidate) to covalently cross-link this IgG to the immobilized protein A. DMP is used as an example, although other homobifunctional cross-linkers, such as glutaraldehyde, could be substituted.



Reagents:

1. ProActive® Protein A coated microspheres (supplied at 1% solids)
2. Antibody Binding Buffer: 50 mM sodium borate, pH 8.2
3. Cross-linking Buffer: 0.2 mM triethanolamine, pH 8.2
4. Quenching Solution: 0.1 M ethanolamine, pH 8.2
5. 1 M NaCl

6. 0.1 M Glycine, pH 2.8
7. Elution Buffer: 0.1 M Glycine-HCl, pH 2.5

Procedure:

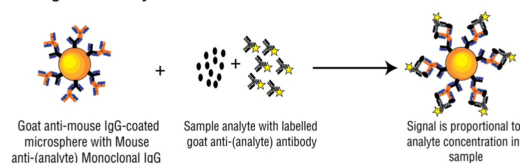
1. Wash 10mL (10mg/mL) Protein A-coated microspheres 2 times in 10mL of antibody binding buffer, remove supernatant after the second wash.
2. Dissolve IgG in up to 10X excess of estimated monolayer amount (based on binding capacity listed on the Certificate of Analysis for Human IgG) in 10mL of antibody binding buffer.
3. Add this antibody suspension to the microsphere pellet, and mix by gently rocking for 45 minutes at room temperature (18-25˚C).
4. *If elution is desired,* as in affinity separation applications, separate microspheres from solution, resuspend microspheres in 10mL of elution buffer for 15 minutes, then wash, separate supernatant, and dialyze this supernatant into desired storage buffer (normally 0.1 M PBS, pH 7.4). *Note:* Separate polymeric and silica microspheres via centrifugation or dialysis, and with a magnet for superparamagnetic microspheres.
5. Separate, discard supernatant, and resuspend in 9mL antibody binding buffer : 1mL cross-linking buffer. Separate and discard supernatant.
6. Dissolve 66mg DMP into 10mL cross-linking buffer. Immediately add this to the microsphere pellet and mix by gentle rocking.
7. React for 1 hour at room temperature.
8. Separate, discard supernatant, and resuspend in 10mL of quenching solution, and allow to react at room temperature for 10 minutes.
9. Wash sequentially in 10mL volumes of the following: 1 M NaCl, 0.1 M glycine (pH 2.8), water.
10. Wash and resuspend in storage buffer (Page 4, *Physical Parameters*) to desired concentration (often 10 mg/mL).
11. Store at 4˚C until used.

IV. ANTI-MOUSE IgG-COATED MICROSPHERES

Secondary antibody-coated microspheres, using proteins such as anti-mouse IgG, are well-suited for many applications where streptavidin- or Protein A-coated microspheres are less than optimal. These have been used in immunoassay and cell separation applications, primarily when capturing antibodies for which other generic binding proteins are inappropriate.[13,14,15,16]

The basis for this technology, and the reason that we use anti-mouse antibodies, is that mouse antibodies, raised against a wide variety of analytes, are readily available. The potential applications of this technology are very broad. The advantage in using a second antibody-coated microsphere is primarily in the ease of antibody attachment. This is done simply by mixing the reagents in the proper concentrations (which can be estimated based on the binding capacity listed on the Certificate of Analysis that we provide with each lot). Our anti-Mouse antibodies are Fc-specific, and bind the heavy chains of all of the mouse IgG subclasses (IgG_1, IgG_{2a}, IgG_{2b}, and IgG_3) with high affinity. By concentrating binding at the Fc region, the Fab regions of the antibodies are directed away from the microspheres, thereby maximizing the biological activities of the antibodies.

Several immunoassay formats incorporate secondary antibody-coated microspheres as the solid support. Here is an example of a sandwich assay format, which relies on some type of detector molecule (fluorescent, radioactive, enzymatic, etc.) to give a quantitative result.[11] Other formats in which this type of microsphere could be used are ELISA, lateral flow, turbidimetric tests and assays, and high throughput screening assays involved in drug discovery.



Magnetic microspheres are a popular choice for separating unbound labelled tracer in this type of application, since they allow the user to separate unbound tracer without centrifugation, allow for easy scale-up automation, and give flexibility in assay configuration.

A. Physical Parameters

Microsphere Types:

Polystyrene...................... 0.02-10.00µm, plain or dyed in a variety of colors, including fluorescent.

Superparamagnetic Polystyrene...................... Polydisperse or monodisperse polystyrene/magnetite with nominal mean diameter of ~1µm.

Two types: Classical, with magnetite exposed at surface, and Encapsulated, with outer polymer shell. Magnetite percentage ranges from 12-66% by weight (density ranges from 1.16-2.24 g/cm^3).

Silica............................... 0.15-5.0µm (density=1.96 g/cm^3)

Concentration: 10mg microspheres/mL (1% solids w/v)

Storage Buffer: 100 mM Borate, pH 8.5 + 0.01% BSA + 0.05% Tween® 20 + 10 mM EDTA + 0.1% NaN_3 (unless otherwise specified).

Binding Capacity: Supplied on the Certificate of Analysis for each lot.

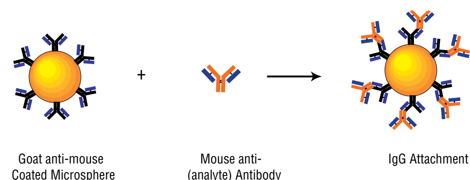
Stability and Storage: Polystyrene and superparamagnetic polystyrene are stable at 2-8˚C for 2 years from date of manufacture, (as determined by accelerated stability testing at 22˚C).

B. Sample Procedures

1. Preparation of Anti-Mouse Coated Microspheres

A preliminary wash is necessary with most applications. This removes various additives, including EDTA, antimicrobials, and surfactants. Several washing methods are possible, and a detailed description of these can be found in our TechNote 203, *Washing Microspheres*. *Note:* ProActive® anti-Mouse coated microspheres are not claimed to be sterile. Therefore, if using these for cell separations in vivo, it is recommended that the microspheres be washed 2-3 times in sterile medium.

2. Attachment/Elution of an Antibody to Anti-Mouse Coated Microspheres



Reagents:

1. ProActive® anti-Mouse coated microspheres (supplied at 1% solids)
2. Wash/Storage Buffer: 0.1 M PBS, pH 7.4
3. Elution Buffer: 0.1 M Glycine-HCl, pH 2.5

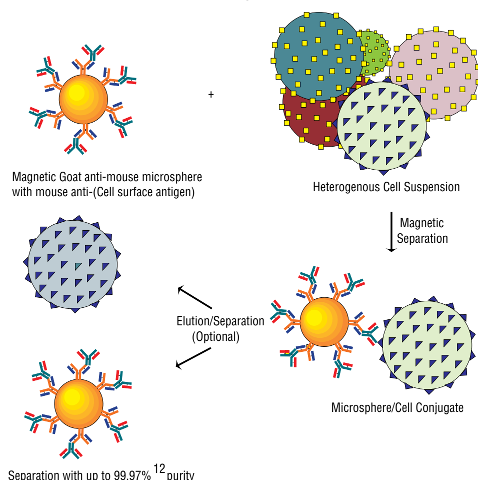
Procedure:
1. Wash an aliquot of particles with a 10X volume of wash buffer.
2. Resuspend the final pellet in wash buffer to a concentration of 0.05% solids (0.5 mg/mL).
3. To this solution, add your antibody dissolved in the same buffer. The protein concentration will have to be optimized, but can be based on the binding capacity of the microspheres, as shown on the Certificate of Analysis.
4. Incubate at room temperature (18-25˚C) for 30 minutes with gentle mixing.
5. Wash the particles 3 times with another 10X volume of wash buffer.
6. Resuspend antibody coated beads in 0.1 M PBS, pH 7.4, to desired storage concentration (often 0.5 mg/mL).
7. *Optional:* Break antibody/antigen interaction by suspending the conjugate in elution buffer for 15 minutes, with gentle mixing, at room temperature. Microspheres can then be pelleted and the supernatant, containing analyte, can be separated for use in your application.

3. Cell Separation

The following protocol is written specifically for anti-mouse coated magnetic microspheres, because of the ease of separation that these offer. Polymeric and silica microspheres can be separated by centrifugation, filtration, or gravimetric settling.

Several factors make our ProActive® anti-Mouse coated magnetic microspheres ideal for cell sorting, including:
1. Excellent specificity for desired cells.
2. Low cost of reagents and equipment to perform separations.
3. Complementary, because of their small size (nominally 1µm), to FACS, or fluorescence activated cell sorters.

The two major approaches to cell separation are positive selection (in which the cells of interest are separated from a heterogeneous population) and negative selection (in which the contaminating cells are removed). The main practical difference between these approaches is that the microspheres are attached to the cells of interest when performing positive selection, and are not attached to the cells of interest in negative selection.



Reagents:
1. Heterogeneous cell suspension
2. Monoclonal mouse anti-(cell surface antigen) IgG
3. ProActive® anti-Mouse IgG-coated magnetic microspheres
4. Elution Buffer: Chymopapain, 200 U/mL. *Note:* The use of chymopapain rather than 0.1 M glycine allows microsphere separation, without damaging the cells.

Procedure:
1. Incubate the heterogeneous mixture of cells with the monoclonal mouse anti-(cell surface antigen) IgG. Use approximately 5-20µg of monoclonal antibody / 10^6 target cells. Allow this incubation to proceed for 30 minutes on ice.
2. Wash to remove unbound antibody.
3. Add washed magnetic particles to the mixture. For negative selection, a ratio of 10-50 particles per cell is often used. For positive selection, a common ratio is 5-10 particles per cell.
4. Mix gently, incubate cells again for 30 minutes on ice.
5. After this incubation, magnetically pellet the microspheres with the cells attached to the side of the vessel (in order to keep unselected cells from contaminating the magnetic pellet due to gravity).
6. *Optional:* Elute microspheres from selected cells by suspending in elution buffer for 10-30 minutes at 37˚C. Pull microspheres to magnet and pour off supernatant-containing cells.

V. REFERENCES

1. **Khosravi, M.** 1995. Application of the biotin-(strept)avidin system in immunochemical techniques. Workshop 111, Clinical Ligand Assay Society Annual Meeting. (May).
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