SPHERO™ Technical Note **STN-12 Rev C 082107**

PROTEIN A COATED MAGNETIC PARTICLES

Protein A is a 42kD polypeptide isolated from Staphylococcus aureus which has a specific binding affinity for the Fc region of IgG from several species. Each Protein A molecule has four IgG binding sites. In addition, protein A binds to IgG without interfering with the antigen binding site of the immunoglobulin. The Protein A coated magnetic particles provide a quick, easy, and economical way for the capture of antigen specific antibodies used in the purification of recombinant antigens.

In the past, protein A linked gel matrix has been routinely used for isolating IgG from human, mouse, and rabbit serum. However, protein A covalently bound to magnetic particles increases the reaction kinetics while reducing the capture time of antigen specific antibodies. As a result, Protein A coated magnetic particles are uniquely suited for isolating IgG from limited volume samples without dilution or loss. They can also be used to capture and concentrate low level IgG in large volume samples. In addition, Protein A coated magnetic particles can be repeatedly used without a significant loss in their ability to bind IgG.

The affinity of Protein A to IgG is not the same for all species as shown below:

Source of IgG	Affinity
Human	Strong
Rabbit	Strong
Mouse	Medium
Rat	Weak
Goat	Weak
Sheep	Weak
Chicken	None

Spherotech offers four Protein A linked magnetic particles for different applications:
SPHERO™ Protein A COATED Magnetic Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Protein A	4.0-4.5	1.0	PAM-40-5	5 mL
Protein A, Smooth Surface	3.0-3.9	1.0	PAMS-30-5	5 mL
Protein A, Smooth Surface	4.0-4.5	1.0	PAMS-40-5	5 mL
Protein A, Cross-linked, granules, non-uniform	~1-2	1.0	PAMX-10-5	5 mL

NOTE: PAMX-10-5 Protein A magnetic particles are recommended for general purpose applications such as absorption and IgG isolation. The other Protein A magnetic particles are better suited for applications such as immunoassays where particles need to be washed and unbound material removed.

The following are some of the applications for Protein A coated magnetic particles. These protocols must be modified to suit individual applications and systems.

Purification of IgG from Hybridoma Tissue Culture for Clone Selection

1. Magnetically separate the particles and remove the supernatant from 100 µL of Protein A particle suspension.
2. Add 100-200 µL of hybridoma fluid to the particle pellet. Shake and mix the suspension.
3. Incubate at room temperature for 10-15 minutes.
4. Magnetically separate the particles and remove the supernatant.
5. Test for the IgG bound to the protein A magnetic particles and select a productive clone.
6. Elute the IgG from the particles with 100 mM glycine, pH 3.0.

NOTE: 100 µL of 1% particles bind 5-8 µg of IgG. Mouse IgG1 and Human IgG3 do not bind well to protein A.

Isolation of Specific Cells from Blood (B, T and HLA) using Protein A Magnetic Particles

1. Bind the Moab to T cells of HLA antigens to the Protein A particles based on their binding capacity.
2. Suspend the white cells rich buffy coat from blood in buffered BSA.
3. Add the Protein A Particle-Antibody complex to the cell suspension.
4. Magnetically separate the particles and remove the supernatant after a short incubation.
5. Wash the particles with buffered BSA solution.
6. Elute the cells captured from the particle-antibody complex with 100 mM glycine, pH 3.0.

NOTE: Protein A will orient the IgG so that the Fab is free to react with the antigen while the Fc remains firmly attached to the particle surface.

All-Purpose "Fish-Hook" for isolating Specific Antigen from a Mixture

A complex of protein A magnetic particles and a specific antibody can be used to capture a specific antigen. The Protein A binds to the antibodies Fc region forming a "Fish-Hook". This complex isolates a specific antigen in a lysate of a prep of tissue culture, recombinant bacteria or yeast culture using the following steps:

1. Add antibody-Protein A particle complex to the cell lysate containing the specific antigen.
2. Incubate briefly while agitating..
3. Magnetically separate the particles.
4. Wash the magnetic particles.
5. Elute the antigen and IgG from the particles with 100 mM glycine, pH 3.0.
6. Separate the IgG and captured antigen chromatographically or by proteolysis.

Polyclonal and Monoclonal Cocktail

Protein A particles complexed with a mixture of monoclonal and polyclonal antibodies maximize antibody affinity and avidity.

Reuse of Protein A Linked Magnetic Particles

At least five cycles of IgG capture and elution can be performed without a significant loss in IgG binding. The following is a suggested protocol for the capture and elution of typical IgG from a sample:

1. Mix and incubate the sample with the particles at room temperature for 10-15 minutes.
2. Separate the particles magnetically or by centrifugation. Collect the supernatant containing unbound proteins.
3. Wash the 2 times using phosphate buffer, pH 7.4 to remove unbound proteins from the particle suspension.
4. Elute the IgG by resuspending the particles in 100 mM glycine, pH 3.0. The IgG will be released into the solution.
5. Regenerate the particles by resuspending them in 0.1 M phosphate buffer, pH 7.4. Repeat 2 times.
6. Store the particles in phosphate buffer, pH 7.4.