

**Protein A Coated Magnetic Particles**

Protein A is a 42 kD polypeptide isolated from *Staphylococcus aureus*, it has specific binding affinity for the Fc region of IgG from several species. There are four IgG binding sites on each Protein A molecule. Protein A binds to IgG from various species selectively and efficiently without interfering with the antigen binding site of the immunoglobulin.

Protein A linked to a gel matrix has been routinely used for isolating IgG from human, mouse and rabbit serum. Protein A covalently bound to magnetic particles further improves efficiency by combining the high affinity of Protein A with the ease of magnetic separation. Fast reaction kinetics of small particles reduces capture time. Protein A coated magnetic particles are uniquely suited for isolating IgG from limited volume samples without dilution or loss and to capture and concentrate low level IgG in large volume sample. Antigen specific antibodies captured by Protein A coated magnetic particles are useful in purification of recombinant antigens in a quick, easy and economical way. Protein A coated magnetic particles can be used repeatedly without a significant loss in their ability to bind IgG.

There are several advantages of using Protein A covalently linked to magnetic particles over Protein A attached to non-magnetic gel or latex. Some are listed below:

1. Magnetic particles coated with Protein A are economical because they can be reused repeatedly without any significant loss in activity.
2. Reaction kinetics of small uniform magnetic particles coated with Protein A is fast and efficient.

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

| <b>Source of IgG</b> | <b>Affinity</b> |
|----------------------|-----------------|
| Human                | Strong          |
| Rabbit               | Strong          |
| Mouse                | Medium          |
| Rat                  | Weak            |
| Goat                 | Weak            |
| Sheep                | Weak            |
| Chicken              | None            |

Protein A coated magnetic particles are available in two forms for different applications:

**Cat. # PAMX-10-5 Non-Uniform Cross-Linked 1-2  $\mu$  Magnetic**

**Cat. # PAM-40-5 Uniform ~ 4  $\mu$  Magnetic**

PAMX-10 Protein A coated magnetic particles are recommended for general purpose applications such as absorption and IgG isolation. PAM-40 Protein A magnetic particles, on the other hand, are better suited for applications such as immunoassays where particles need to be washed and unbound material removed.

Following are a few of the many applications of Protein A coated magnetic particles. The protocols are tentative and must be modified to suit individual applications and systems.

### **# 1 Purification of IgG from Hybridoma Tissue Culture For Clone Selection**

1. Magnetically separate particles from 100  $\mu$ L of Protein A particle suspension; remove supernatant.
2. Add 100-200  $\mu$ L of hybridoma fluid to the particle pellet. Shake and mix the suspension.
3. Incubate at room temperature for 10-15 minutes.
4. Magnetically separate particles and remove supernatant.
5. IgG bound to the Protein A on the magnetic particles can be tested for the IgG and select a productive clone.
6. IgG from the particles can be eluted by treating it with 100 mM glycine, pH 3.0.

Note:

1. 100  $\mu$ L of 1% particles will bind 5-8  $\mu$ g of IgG.
2. Mouse IgG type 1 and Human IgG type 3 do not bind well to Protein A.

### **# 2: All-Purpose "Fish-Hook" for Isolating Specific Antigen from a Mixture.**

Form a complex by mixing Protein A coated magnetic particles with a specific antibody. Protein A binds to the Fc of the antibody forming a "Fish-Hook" to capture specific antigen. To isolate a specific antigen present in a lysate of a prep of tissue culture, recombinant bacteria or yeast culture follow the following steps.

1. To the cell lysate containing the specific antigen, add antibody-Protein A particle complex.
2. Incubate briefly while agitating.
3. Magnetically isolate particles.
4. Wash particles.
5. Elute antigen and IgG from particles by treating with 100 mM glycine, pH 3.0.
6. IgG and captured antigen may be separated either chromatographically or by proteolysis of IgG using magnetic particles linked Papain (PAPM-40), Pepsin (PEPM-40), or Trypsin (TRPM-40) available from Spherotech.

**# 3: Isolation of Specific Cells from Blood (B, T and HLA) Using Protein A Coated Magnetic Particles**

Protein A particles can be an easy way to isolate cells. Since we know the exact amount of IgG a given particle will bind to, we can minimize waste of antibodies. Moreover, Protein A allows a convenient way to orient the IgG in an effective way i.e. Fab free to react with antigen while Fc is remaining firmly attached to the solid surface of particles. Protein A magnetic particles may be used for the isolation of B cells, T cells or specific HLA Type cells from blood. White cells rich buffy coat from blood is suspended in buffered BSA. Protein A Particle complexed with Moab to T cells or HLA antigens are added to the cell suspension. After a short incubation particles are magnetically separated and supernatant removed. Particles are washed with buffered BSA solution. Cells captured by Protein A particle-antibody complex are eluted with the help of 100 mM glycine, pH 3.0.

**# 4: Polyclonal and Monoclonal Cocktail**

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

**Reuse Protein A Coated Magnetic Particles**

IgG from mouse serum mixed with these particles can be eluted using 100 mM glycine, pH 3.0. At least five cycles of IgG capture and elution are easily carried out without a significant loss in IgG binding capacity yielding comparable yields at each elution cycle. The following is a suggested format for capture and elution of typical IgG from a sample:

1. Mix and incubate sample with particles; 10-15 min. at room temperature.
2. Separate particles (magnetically/centrifugation); collect supernatant (unbound proteins).
3. Wash particles 2 X with phosphate buffer, pH 7.4; removes remaining unbound proteins from the particle suspension.
4. Elute IgG by resuspending particles in 100 mM glycine, pH 3.0; IgG is released into the solution.
5. Regenerate particles by resuspending in 0.1 M phosphate buffer, pH 7.4. Repeat 2 X.
6. Store particles for next capture by resuspending in phosphate buffer, pH 7.4 as in the original volume of particles.