PureProteome™ Protein A and Protein G Magnetic Beads User Guide

Introduction

The Fc portion of a variety of immunoglobulins (Ig) is known to bind to several bacterial proteins, including Protein A from *Staphylococcus aureus* and Protein G, which is produced by various *Streptococcus* species. The binding affinities of immunoglobulins for Protein A and Protein G vary depending on both the species used to generate the antibody, as well as the antibody's isotype (see Table 1 for details). Both Protein A and Protein G can be extremely useful research tools for applications involving antibodies.

PureProteome Protein A and Protein G Magnetic Beads further enhance the utility of Protein A and Protein G by covalently coupling these reagents to paramagnetic affinity media (i.e., magnetic beads). These beads provide users with a bench-top platform for the rapid, reproducible separation of immunoglobulins from complex mixtures such as serum samples, tissue culture supernatants, and cellular lysates. To achieve separation, samples are mixed with PureProteome Protein A or Protein G Magnetic Beads for a short period of time to bind the immunoglobulins. The beads are then isolated using a magnetic stand, followed by several wash steps to remove unbound proteins. Finally, the bound proteins are eluted at high purity. This magnetic system is a convenient format for serum depletion, immunoprecipitation, or other applications that employ Protein A or Protein G.

Materials Required to Use PureProteome Protein A and Protein G Magnetic Beads

For optimal performance, the Millipore PureProteome Magnetic Stand is recommended for use with PureProteome Protein A and Protein G Magnetic Beads.

Application Guidelines

Immunoglobulin Depletion from Serum Samples

Proteomic analysis of complex biological samples such as serum or plasma is hindered by the presence of highly abundant proteins, including immunoglobulins and albumin. The high binding capacity of the PureProteome Protein A and Protein G Magnetic Beads provides a rapid, scalable, and reproducible means to deplete immunoglobulins from serum and plasma samples, allowing detection and analysis of low abundant proteins.

1. Based on the information in Table 1, select either PureProteome Protein A or Protein G Magnetic Beads.
2. Gently mix the bead suspension so that all of the beads are uniformly resuspended.
3. Pipette 100 µL of the suspended beads into a 1.5 mL microcentrifuge tube.

Table 1. Relative Affinity of Protein A and Protein G (see references on page 4)

Key code for relative affinity of Protein A and G for respective antibodies:

++ = Strong affinity + = Moderate/slight affinity +/– = Requires evaluation – = No affinity

	Protein A	Protein G		Protein A	Protein G		Protein A	Protein G
Human IgG_1	++	++	Mouse IgM	+/–	–	Sheep IgG	+/–	+
Human IgG_2	++	++	Rat IgG	++	++	Goat IgG	+/–	+
Human IgG_3	–	++	Rat IgG_1	+/–	+	Pig IgG	++	++
Human IgG_4	++	++	Rat IgG_{2a}	+/–	++	Chicken IgG	–	+/–
Human IgA	+	–	Rat IgG_{2b}	+/–	+	*Fragments*		
Human IgD	+	–	Rat IgG_{2c}	+/–	+	Human Fab	+	+
Human IgE	+	–	Rat IgM	+/–	–	Human F(ab')2	+	+
Human IgM	+	–	Rabbit IgG	++	++	Human scFv	+	–
Mouse IgG_1	+	+	Hamster IgG	+	++	Human Fc	+	+
Mouse IgG_{2a}	++	++	Guinea Pig IgG	++	+	Human κ	–	–
Mouse IgG_{2b}	++	++	Bovine IgG	+	+	Human λ	–	–
Mouse IgG_3	+	++						

Immunoglobulin Depletion, continued

4. Place the tube into the magnetic stand, allow the beads to migrate to the magnet, and then remove the storage buffer with a pipette.
5. Wash the beads by adding 500 µL of PBS and vortexing vigorously for 10 seconds. Return the tube to the magnetic stand and allow the beads to migrate to the magnet. Remove the buffer with a pipette.
6. Dilute 10–25 µL of serum to a final volume of 100–200 µL with PBS.
7. Add the diluted serum sample to the beads. Incubate for 30 minutes at room temperature with continuous mixing.
8. Place the tube back into the magnetic stand. Allow the beads to migrate to the magnet. Remove the supernatant with a pipette and save. This represents the depleted serum sample.

To elute the bound Ig from the beads continue with the steps below.

1. Wash the beads 3 times using 500 µL of PBS for each wash.
2. Remove the tube from the magnetic stand, add 50 µL of 0.2 M Glycine (pH 2.5). Vortex to mix.
3. Allow the sample to incubate at room temperature for 2 minutes.
4. Place the tube back into the magnetic stand. Allow the beads to migrate to the magnet. Remove the supernatant with a pipette and save.
5. Repeat elution if desired.
6. To neutralize the pH, add 5 µL of 1 M Tris (pH 8.5).

Immunoprecipitation

PureProteome Protein A and Protein G Magnetic Beads are ideally suited for immunoprecipitation (IP) reactions. There are two main methods commonly used for immunoprecipitation: direct and indirect. In direct IP, the capture antibody is first immobilized onto the PureProteome Protein A or Protein G Magnetic Bead, generating an immunoaffinity magnetic bead. These antibody-coupled beads are then added to the sample (e.g., cell lysate) so that the bead-bound antibody can capture the antigen or protein complex of interest. In contrast, for indirect IP, the capture antibody is first incubated with the sample to allow formation of the antibody-antigen complex in solution. The PureProteome Protein A or Protein G Magnetic Beads are then incubated with the pre-formed antibody-antigen complex for capture onto the beads.

While both approaches are suitable for use with PureProteome Protein A or Protein G Magnetic Beads, the choice of approach is typically one of preference. There may be instances where one approach results in superior IP performance or offers greater convenience. For example, the direct IP approach could be used for the bulk preparation of immunoaffinity magnetic beads for future use, whereas the indirect method may be preferred where there are concerns about steric hindrance during the antigen-antibody binding step. Optimization of the specific immunoprecipitation reaction is recommended prior to finalizing the method. Because of the large variability in antibody affinity/avidity, no single protocol can provide optimal IP results in all cases. Parameters that may need to be optimized by the user include the amount of capture antibody used, sample concentration and preparation method, as well as incubation

Immunoprecipitation, continued

time and temperature. The following protocol is intended as a starting point for developing an optimized protocol.

Direct Immunoprecipitation Protocol

1. Based on the information in Table 1, select either PureProteome Protein A or Protein G Magnetic Beads.
2. Gently mix the bead suspension so that all of the beads are uniformly resuspended.
3. Pipette 50 µL of suspended beads into a 1.5 mL microcentrifuge tube.
4. Place the tube into the magnetic stand, allow the beads to migrate to the magnet, and then remove the storage buffer with a pipette.
5. Wash the beads by adding 500 µL of PBS containing 0.1% Tween® 20 surfactant and vortexing vigorously for 10 seconds. Return the tube to the magnetic stand and allow the beads to migrate to the magnet. Remove the buffer with a pipette.
6. Resuspend the washed beads in 100 µL of PBS containing 0.1% Tween 20 surfactant.
7. Add the capture antibody to the resuspended beads.
8. Incubate at room temperature for 10 minutes with continuous mixing.
9. Place the tube into the magnetic stand, allow the beads to migrate to the magnet, and then remove the buffer with a pipette.
10. Wash the beads 3 times with 500 µL of PBS containing 0.1% Tween 20 surfactant.
11. After the last wash, remove the tube from the stand and add the sample.
12. Incubate the sample and immobilized capture antibody at 2–8 °C with continuous mixing. Refer to the antibody manufacturer's recommendations for the capture antibody concentration and incubation time. Times may vary from a few hours to overnight.
13. Place the tube into the magnetic stand, allow the beads to migrate to the magnet, and then remove the sample with a pipette.
14. Wash the beads 3 times with 500 µL of PBS containing 0.1% Tween 20 surfactant.
15. After the last wash, remove the tube from the magnetic stand and add the appropriate elution buffer for denaturing or native elution.

    Denaturing Elution: Add 60 µL of sample buffer suitable for electrophoresis and mix to resuspend the beads. Heat at 70–90 °C for 10 minutes. Place the tube into the magnetic stand, allow the beads to migrate to the magnet, and immediately transfer the supernatant to a new tube with a pipette.

    Native Elution: Add 60 µL of 0.2 M Glycine (pH 2.5), mix to resuspend the beads, then incubate for 1–2 minutes at room temperature. Place the tube into the magnetic stand and allow the beads to migrate to the magnet. Transfer the supernatant to a new tube with a pipette and neutralize by adding 5 µL of 1 M Tris (pH 8.5).

    NOTE: Smaller elution volumes (minimum 20 µL) can be used, however, yields will be slightly lower. To achieve maximum yield with a smaller elution volume, a second elution is recommended.

Indirect Immunoprecipitation Protocol

1. Incubate the sample and capture antibody at 2–8 °C with continuous mixing. Refer to the antibody manufacturer's recommendations for the capture antibody concentration and incubation time. Times may vary from a few hours to overnight.
2. Based on the information in Table 1, select either PureProteome Protein A or Protein G Magnetic Beads.
3. Gently mix the bead suspension so that all of the beads are uniformly resuspended.
4. Pipette 50 µL of suspended beads into a clean 1.5 mL microcentrifuge tube.
5. Place the tube into the magnetic stand, allow the beads to migrate to the magnet, and then remove the storage buffer with a pipette.
6. Wash the beads by adding 500 µL of PBS containing 0.1% Tween 20 surfactant and vortexing vigorously for 10 seconds. Return the magnetic beads to the stand and allow them to migrate to the magnet. Remove the buffer with a pipette.
7. Add the reaction containing the pre-formed antibody-antigen complex (from step 1) to the beads.
8. Incubate for 10 minutes at room temperature with continuous mixing to capture the immune complex.
9. Follow steps 13 through 15 in the Direct Immunoprecipitation Protocol.

Disposal

Used material may be discharged into sewer or industrial waste water systems if allowed by local regulations. Otherwise, collect and dispose according to federal, state, and local regulations.

Specifications

Matrix	Polymer-coated inorganic beads with covalently coupled recombinant Protein A or Protein G
Particle form	Spherical
Bead diameter	10 µm (nominal)
Storage	2–8 °C. Do not freeze.
Capacity*	
Protein A	Typically 1.5–2.5 mg of rabbit IgG per mL of suspension (25 mg/mL settled beads)
Protein G	Typically 2.5–3.5 mg of rabbit IgG per mL of suspension (35 mg/mL settled beads)

PureProteome Protein A and Protein G Magnetic Beads are for research use only.

** Performance will vary depending on the antibody isotype and/or species of the organism used to generate the antibody. Refer to Table 1.*

Product Ordering Information

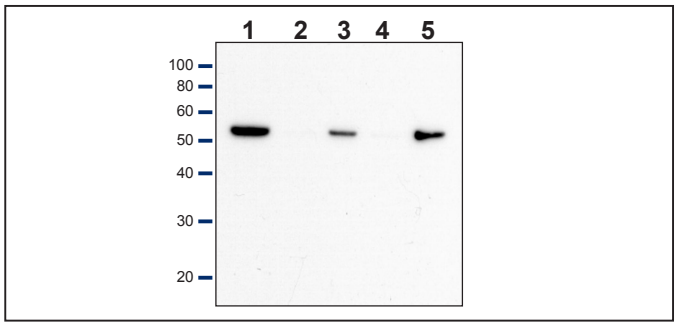
Description	Qty/Pk	Cat. No.
PureProteome Protein A Magnetic Beads	2 x 1 mL 1 x 10 mL	LSKMAGA02 LSKMAGA10
PureProteome Protein G Magnetic Beads	2 x 1 mL 1 x 10 mL	LSKMAGG02 LSKMAGG10
PureProteome Magnetic Stand, 8-well	1	LSKMAGS08
PureProteome Magnetic Stand, 15 mL	1	LSKMAGS15

Performance

Figure 1. Immunodepletion of Immunoglobulins From Rabbit Serum using PureProteome Protein A and Protein G Magnetic Beads

Rabbit serum (10 µL) was diluted with 190 µL of PBS and the immunoglobulins were depleted using either PureProteome Protein A (lanes 2–4) or Protein G (lanes 7–9) Magnetic Beads. Samples of the input material, flow through, and bound fractions were separated by SDS-PAGE and stained with colloidal Coomassie blue. Lanes 1 and 6 show molecular weight markers, 2 and 7 show the input material, 3 and 8 show the flow through Ig depleted serum, and 4 and 9 show the bound Ig fraction. ELISA assay results revealed 98% Ig depletion for both Protein A and Protein G.

Figure 2. Immunoprecipitation of p53 from A431 Cell Lysates Using PureProteome Protein A and Protein G Magnetic Beads



Whole-cell lysates (500 µg of protein) prepared from human epidermoid carcinoma (A431) cells were incubated with 5 µg of mouse monoclonal anti-p53 antibody. PureProteome Protein A (lanes 2 and 3) or Protein G Magnetic Beads (lanes 4 and 5) were then added to the reaction to capture the immune complexes following the indirect immunoprecipitation protocol. Samples of input material (lane 1), flow through (lanes 2 and 4) and bound fractions (lanes 3 and 5) were separated by SDS-PAGE prior to semi-dry transfer to Immobilon®-P blotting membrane. Immunodetection was performed with the SNAP i.d.® Protein Detection System using a rabbit polyclonal anti-p53 antibody (diluted 1:1,000) and a horseradish peroxidase conjugated goat anti-rabbit secondary antibody (diluted 1:40,000). The blot shown was visualized using Immobilon® Western HRP Substrate and represents a 1-minute exposure to x-ray film.

Troubleshooting/Optimization

Problem: Low Immunoglobulin binding	
Cause	**Solution**
Insufficient bead volume	Ensure that the beads are well suspended prior to pipetting. Mix the beads while pipetting.
Insufficient mixing	Mix beads and sample continuously with either a vortex mixer or end-over-end mixing.
Incorrect bead	Refer to Table 1 to match the host and isotype of Ig with either PureProteome Protein A or Protein G Magnetic Beads.
Insufficient incubation	Optimization may be required. A 10-minute incubation is recommended as a starting point, but this is dependent on the sample volume and affinity of antibody for target antigen.
Problem: High background	
Cause	**Solution**
Insufficient washing	Wash the beads at least 3 times with PBS containing 0.1% Tween 20 surfactant prior to eluting the sample. Ensure complete removal of buffer.
Problem: Magnetic beads do not collect on the magnet	
Cause	**Solution**
Magnet strength not sufficient	Use the PureProteome Magnetic Stand for optimal performance. Make sure the tube is in contact with the magnet.
Problem: Poor recovery	
Cause	**Solution**
Incorrect elution volume	Elute the sample in volumes between 20 and 60 µL. If the target is a low abundant protein, smaller elution volumes are recommended. Perform a second elution.

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