User Guide

PureProteome™ Protein A/G Mix Magnetic Beads

Cat. Nos. LSKMAGAG02 and LSKMAGAG10

Introduction

PureProteome™ Protein A/G Mix Magnetic Beads combine the immunoglobulin binding affinities of Protein A from *Staphylococcus aureus* and Protein G from *Streptococcus* in one convenient magnetic bead product. Since the immunoglobulin binding affinities of Protein A and Protein G vary depending on the host species used to generate the antibody and the antibody's isotype (see Table 1), the combination of the two ligands results in greater antibody compatibility than either Protein A or Protein G alone.

The magnetic beads provide users with a bench-top platform for the rapid, reproducible capture of immunoglobulins from complex mixtures such as serum samples, tissue culture supernatants, and cell lysates. To achieve capture, samples are mixed with PureProteome™ Protein A/G Mix Magnetic Beads to bind the immunoglobulins. The beads are then isolated using a magnetic stand, followed by wash steps to remove unbound proteins. Finally, the bound proteins are eluted at high purity. In immunoprecipitation experiments, co-elution of the capture antibody can obscure detection of the target protein; in these cases crosslinking of the antibody to the PureProteome™ Protein A/G Mix Magnetic Beads eliminates elution of the capture antibody along with the target. This magnetic bead system is a convenient format for immunoprecipitation and other applications that utilize Protein A and/or G for target capture.

Application Guidelines

Immunoprecipitation

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

Application Guidelines, continued

While both approaches are suitable for use with PureProteome™ Protein A/G Mix Magnetic Beads, the choice of approach is typically one of preference. There may be instances where one approach results in superior immunoprecipitation performance or offers greater convenience. Optimization of the specific immunoprecipitation protocol is recommended. Because of variability in antibody affinity/avidity, no single protocol can provide optimal immunoprecipitation results in all cases. Parameters that may need to be optimized by the user include the amount of capture antibody used, sample preparation method and concentration, incubation time and temperature, and wash buffer constituents. The following protocols are intended as a starting point for developing an optimized protocol.

Relative Affinity of Protein A/G Mix for Various Antibodies

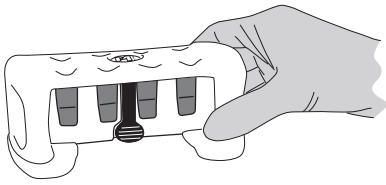
Table 1. Key for relative affinity (see references on page 6)

++ Strong affinity		+/– Requires evaluation	
+ Moderate/slight affinity		– No affinity	
Antibody	**Affinity**	**Antibody**	**Affinity**
Human IgG	++	Rat IgG_{2b}	+
Human IgG_1	++	Rat IgG_{2c}	+
Human IgG_2	++	Rat IgM	+/–
Human IgG_3	++	Rabbit IgG	++
Human IgG_4	++	Hamster IgG	++
Human IgA	+	Guinea pig IgG	++
Human IgD	+	Bovine IgG	+
Human IgE	+	Sheep IgG	+
Human IgM	+	Goat IgG	+
Mouse IgG	++	Pig IgG	++
Mouse IgG_1	+	Chicken IgG	+/–
Mouse IgG_{2a}	++	Fragments*	
Mouse IgG_{2b}	++	Human Fab	+
Mouse IgG_3	++	Human F(ab')2	+
Mouse IgM	+/–	Human scFv	+
Rat IgG	+	Human Fc	+
Rat IgG_1	+	Human κ	–
Rat IgG_{2a}	++	Human λ	–

* Refer to PureProteome™ Kappa and Lambda Magnetic Beads for affinity with fragments.

Tips for Using the PureProteome™ Magnetic Stand

For optimal performance, the PureProteome™ Magnetic Stand is recommended for use with PureProteome™ Magnetic Beads.



The magnetic stand facilitates immunoprecipitation procedures by capturing the magnetic beads so that buffers, antibodies, and other reagents can easily be added and removed. For many of the protocol steps, the addition and mixing of components can be done with the tubes remaining in the stand.

- When instructed to "wash the beads", first disengage the magnet bar and set it aside. Add the appropriate buffer and vortex the samples by placing the stand on a vortex equipped with a 3 inch platform. **Hold stand firmly!**

  Note: During mixing, magnetic beads may accumulate in the microcentrifuge tube cap. These beads can be recovered with a pulse in a microcentrifuge, or by engaging the magnet bar in the stand and inverting the stand to "wash down" beads left in the cap.
- When instructed to "capture the beads", re-engage the magnet bar by sliding it up into the stand until it clicks into place.

Protocol A – Indirect Immunoprecipitation

Materials Required but Not Supplied

- 1.5 mL microcentrifuge tubes
- PureProteome™ Magnetic Stand (8-well), cat. no. LSKMAGS08
- Binding/wash buffer: Phosphate-buffered saline (PBS) containing 0.01–0.1% Tween® 20 detergent, pH 7.4
- Elution Buffer:

  Native elution: 0.2 M Glycine-HCl, pH 2.5

  Denaturing elution: 1X SDS-PAGE sample loading buffer suitable for electrophoresis, containing dithiothreitol (DTT) or β-mercaptoethanol
- Neutralization Buffer: 1 M Tris-HCl, pH 8.5
- Capture antibody: Typically 4–10 µg per reaction

1. Incubate the sample and capture antibody at 2–8 °C or room temperature 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. Gently mix the PureProteome™ Protein A/G Mix Magnetic Bead suspension so that all of the beads are uniformly resuspended.
3. Pipette 25–50 µL of suspended beads into a clean 1.5 mL microcentrifuge tube.
4. Place the tube into the magnetic stand to capture the beads, then remove the storage buffer with a pipette and discard.

Protocol A – Indirect Immunoprecipitation, continued

5. Disengage the magnet from the stand and wash the beads for 10 seconds with 500 µL of binding/wash buffer. Re-engage the magnet to capture the beads, then remove the buffer with a pipette and discard.
6. Disengage the magnet and add the preformed antibody-antigen complex (from step 1) to the beads.
7. Incubate for 10–30 minutes at room temperature with continuous mixing to capture the immune complex.
8. Re-engage the magnet to capture the beads, then remove the sample with a pipette.
9. Disengage the magnet and wash the beads for 10 seconds with 500 µL of binding/wash buffer. Repeat 2 more times for a total of 3 washes.
10. After the last wash, disengage the magnet and add the appropriate elution buffer for native or denaturing elution.

    Native Elution: Add 60 µL of 0.2 M Glycine-HCl, pH 2.5, mix to resuspend the beads, then incubate with mixing for 1–2 minutes at room temperature. Re-engage the magnet to capture the beads. Transfer the supernatant to a new tube with a pipette, and neutralize by adding 5 µL of 1 M Tris-HCl, pH 8.5.

    Denaturing Elution: Add 60 µL of elution buffer suitable for electrophoresis, and mix to resuspend the beads. Heat at 70 °C for 10 minutes (elution conditions vary based on buffer system used; refer to manufacturer's recommendation). Re-engage the magnet to capture the beads and immediately transfer the supernatant to a new tube with a pipette.

    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.

Protocol B – Direct Immunoprecipitation

Materials Required but Not Supplied

- 1.5 mL microcentrifuge tubes
- PureProteome™ Magnetic Stand (8-well), cat. no. LSKMAGS08
- Binding/wash buffer: Phosphate-buffered saline (PBS) containing 0.01–0.1% Tween® 20 detergent, pH 7.4
- Elution Buffer:

  Native elution: 0.2 M Glycine-HCl, pH 2.5

  Denaturing elution: 1X SDS-PAGE sample loading buffer suitable for electrophoresis, containing dithiothreitol (DTT) or β-mercaptoethanol
- Neutralization buffer: 1 M Tris-HCl, pH 8.5
- Capture antibody: Typically 4–10 µg per reaction

1. Gently mix the PureProteome™ Protein A/G Mix Magnetic Bead suspension so that all of the beads are uniformly resuspended.
2. Pipette 25–50 µL of suspended beads into a 1.5 mL microcentrifuge tube.
3. Place the tube into the magnetic stand to capture the beads, then remove the storage buffer with a pipette and discard.

Protocol B – Direct Immunoprecipitation, continued

4. Disengage the magnet from the stand and wash the beads for 10 seconds with 500 µL of binding/wash buffer. Re-engage the magnet to capture the beads, then remove the buffer with a pipette and discard.
5. Disengage the magnet and resuspend the washed beads in 100 µL binding/wash buffer.
6. Refer to the antibody manufacturer's recommendations for the capture antibody concentration and add the capture antibody to the resuspended beads.
7. Incubate at room temperature for 10–30 minutes with continuous mixing.
8. Re-engage the magnet to capture the beads, then remove the buffer with a pipette and discard.
9. Disengage the magnet and wash the beads for 10 seconds with 500 µL of binding/wash buffer. Repeat 2 more times for a total of 3 washes.

   Note: Beads are now ready for immunoprecipitation or antibody crosslinking (refer to Protocol C).
10. After the last wash, disengage the magnet and add the sample.
11. Incubate the sample and magnetic beads with immobilized antibody at room temperature or 2–8 °C with continuous mixing. Incubation times may vary from a few hours to overnight (refer to antibody manufacturer's recommendations).
12. Follow steps 8–10 of Protocol A - Indirect Immunoprecipitation.

Protocol C – Antibody Crosslinking Using Bis(sulfosuccinimidyl) suberate (BS^3)

Note: Dimethyl pimelimidate (DMP) and disuccinimidyl suberate (DSS) have also been used successfully for crosslinking. Refer to data sheet PC5522EN00 or PC5522ENEU.

Crosslinker solution should be prepared immediately before use and should not be stored for later use.

Materials Required but Not Supplied

- PureProteome™ Magnetic Stand (8-well), cat. no. LSKMAGS08
- Milli-Q® water or equivalent
- Bis(sulfosuccinimidyl) suberate (BS^3) crosslinker
- Crosslink buffer: 20 mM Sodium phosphate, 0.15 M NaCl, pH 7–9
- Wash/storage buffer: Phosphate-buffered saline (PBS) with 0.01–0.05% Tween® 20 detergent, pH 7.4
- Quench buffer: 1 M Tris-HCl, pH 7.5
- 0.2 M Glycine-HCl, pH 2.5
- Antibody: Typically 4–10 µg per reaction

1. Follow antibody binding protocol outlined in steps 1–9 of the Protocol B - Direct Immunoprecipitation.
2. After the last wash, disengage the magnet. Wash the beads for 1 minute with 500 µL of crosslink buffer. Re-engage the magnet to capture the beads, then remove buffer with a pipette and discard. Disengage the magnet.

Protocol C – Antibody Crosslinking Using BS^3, continued

3. Each crosslinking reaction requires 250 µL of 5 mM cross-linker solution. Prepare a 100 mM BS^3 stock solution by weighing out 2 mg of BS^3 and dissolving in 35 µL of Milli-Q® water. Once dissolved, dilute to 5 mM by adding 665 µL of crosslink buffer.
4. Add 250 µL of 5 mM BS^3 solution to each crosslinking reaction. Incubate with end-over-end mixing for 30–60 minutes at room temperature.
5. To quench the crosslinker, add 12.5 µL of quench buffer to each reaction and incubate for 30–60 minutes at room temperature with end-over-end mixing.
6. Re-engage the magnet to capture the beads, then remove the solution with a pipette and discard. Disengage the magnet.
7. To remove any non-crosslinked antibody, wash beads for 1 minute with 500 µL of 0.2 M Glycine-HCl, pH 2.5. Re-engage the magnet to capture the beads, then remove wash solution with a pipette and discard.
8. Wash beads for 1 minute with 500 µL wash/storage buffer. Repeat 2 more times for a total of 3 washes.
9. Use the beads immediately in the immunoprecipitation experiment or store at 4° C. For long term storage, addition of a bacteriostat such as sodium azide (0.05%) is recommended to prevent microbial growth.

Protocol D – Antibody Purification

PureProteome™ Protein A/G Mix Magnetic Beads are suited for antibody purification from a variety of samples. Bead slurry volumes should be optimized for the amount of antibody present in the sample. Very dilute samples may be concentrated using Amicon® Ultra concentrators, while concentrated samples may require dilution to obtain optimum performance.

Materials Required but Not Supplied

- 1.5 mL microcentrifuge tubes
- PureProteome™ Magnetic Stand (8-well), cat. no. LSKMAGS08
- Binding/wash buffer: Phosphate-buffered saline (PBS) or PBS containing 0.01–0.1% Tween® 20 detergent, pH 7.4
- Elution buffer: 0.2 M Glycine-HCl, pH 2.5
- Neutralization buffer: 1 M Tris-HCl, pH 8.5
- Sample: Serum, cell culture supernatant

1. Gently mix the PureProteome™ Protein A/G Mix Magnetic Bead suspension so that all of the beads are uniformly resuspended.
2. Determine the required amount of magnetic bead slurry based on the antibody concentration in the sample. Pipette resuspended bead slurry into a 1.5 mL microcentrifuge tube.
3. Place the tube into the magnetic stand to capture the beads, then remove the storage buffer with a pipette and discard.
4. Disengage the magnet from the stand and wash the beads for 10 seconds with 500 µL of binding/wash buffer. Re-engage the magnet to capture the beads, then remove the buffer with a pipette and discard.
5. If necessary, dilute sample with binding/wash buffer. As a guide, the minimum reaction volume during antibody capture should be equal to the volume of bead slurry used.

Protocol for Antibody Purification, continued

6. Disengage the magnet and add the sample to the beads. Incubate for 30–60 minutes at room temperature with continuous mixing.
7. Re-engage the magnet to capture the beads, then remove the unbound fraction with a pipette and save if further analysis is required.
8. Wash the beads for 30 seconds with 500 µL of binding/wash buffer. Repeat 2 more times for a total of 3 washes.
9. Disengage the magnet and add 50–100 µL of 0.2 M Glycine-HCl, pH 2.5.
10. Allow the sample to incubate at room temperature for 5 minutes with mixing.
11. Re-engage the magnet to capture the beads, then remove the eluted fraction and save.
12. Repeat elution if desired.
13. To neutralize the pH, add 5–10 µL of 1 M Tris-HCl, pH 8.5.

Troubleshooting/Optimization

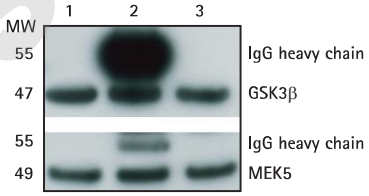
Problem: Low immunoglobulin binding	
Cause	**Solution**
Insufficient bead volume	Ensure that the beads are well suspended prior to pipetting. Mix the beads while pipetting.
Insufficient mixing	Mix beads and sample continuously with either a vortex mixer or end-over-end mixing.
Insufficient incubation	Optimization may be required. A minimum of 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 0.01–0.1% Tween® 20 detergent prior to eluting the sample. Ensure complete removal of buffer. Increase wash cycle time.
Prolonged incubation time	Use Indirect Immunoprecipitation Protocol A.
Nonspecfic interaction	Increase NaCl concentration in wash buffer to reduce nonspecific ionic interactions. NaCl can be increased up to 1 M, but actual concentration requires optimization. Depending on downstream analysis, beads may be blocked with 1–2% bovine serum albumin (BSA).

Troubleshooting/Optimization, continued

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 100 µL. If the target is a low abundant protein, smaller elution volumes are recommended. Perform a second elution.

Performance

Figure 1. Immunoprecipitation with and without crosslinking of capture antibody to PureProteome™ Protein A/G Mix Magnetic Beads

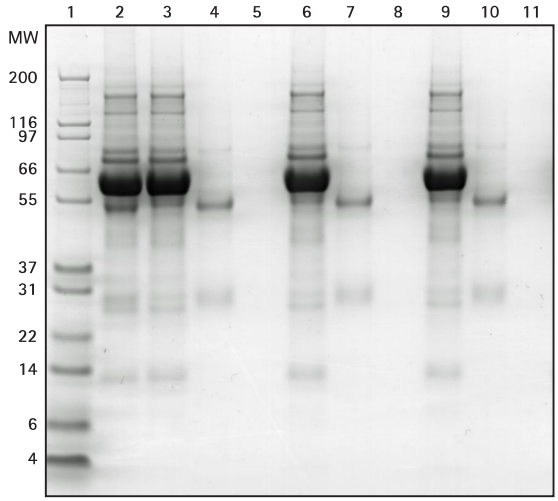


Lane 1	Lane 2	Lane 3
Rat brain lysate	Traditional immuno-precipitation	Crosslinked immuno-precipitation

Primary antibody was bound to PureProteome™ Protein A/G Mix Magnetic Beads. The beads were then used in immunoprecipitation experiments with and without further BS^3 crosslinking. Rat brain lysate was incubated with the magnetic beads for target protein capture. The beads were washed and the target was removed by denaturing elution. Samples from the traditional and crosslinked immunoprecipitation experiments as well as the rat brain lysate input were separated by SDS-PAGE prior to semi-dry transfer onto Immobilon® P Blotting Membrane. Immunodetection was performed using the SNAP i.d.® 2.0 Protein Detection System. The same primary antibody used for immunoprecipitation was used for immunodetection. Bands were visualized using Luminata™ Forte Chemiluminescent Detection Reagent. The antibody-crosslinked PureProteome™ Protein A/G Mix Magnetic Beads successfully captured the target, and the crosslinking eliminated the antibody contamination present in traditional immunoprecipitation experiments.

Performance, continued

Figure 2. Antibody purification from rabbit serum using PureProteome™ Protein A/G Mix Magnetic beads



Lane 1	Lane 2	Lanes 3, 6, 9	Lanes 4, 7, 10	Lanes 5, 8, 11
Molecular weight markers	Input	Unbound fraction	Eluted fraction	Stripped fraction

Antibody purification from rabbit serum was carried out in triplicate using PureProteome™ Protein A/G Mix Magnetic Beads. Elution of the antibody was performed using 0.2 M Glycine-HCl, pH 2.5, and any residual protein was stripped from the magnetic beads using 1X SDS-PAGE reducing sample loading buffer. Samples were separated by SDS-PAGE and bands were visualized by Coomassie staining.

Specifications

PureProteome™ Protein A/G Mix Magnetic Beads	
Matrix	Mixture of polymer-coated magnetic silica beads with covalently coupled recombinant Protein A and beads with covalently coupled recombinant Protein G in 1% benzyl alcohol
Particle form	Spherical
Bead diameter	10 µm (nominal)
Storage	2–8 °C. Do not freeze.
Binding capacity	Typically binds 2–3 mg rabbit IgG per mL slurry
Shelf life	Refer to expiration date on product label

PureProteome™ Protein A/G Mix Magnetic Beads are for research use only. They are not for use in diagnostic procedures.

Disposal

Collect and dispose of used material according to all applicable international, federal, state, and local regulations.

Safety Data Sheet

Safety Data Sheets (SDS) are available on our web site. Go to www.millipore.com and enter your catalogue number in the search box.

Product Ordering Information

Description	Qty/Pk	Cat. No.
PureProteome™ Protein A/G Mix Magnetic Beads	2 × 1 mL 1 × 10 mL	LSKMAGAG02 LSKMAGAG10
PureProteome™ Protein A Magnetic Beads	2 × 1 mL 1 × 10 mL	LSKMAGA02 LSKMAGA10
PureProteome™ Protein G Magnetic Beads	2 × 1 mL 1 × 10 mL	LSKMAGG02 LSKMAGG10
PureProteome™ Kappa Ig Binder Magnetic Beads	2 mL	LSKMAGKP02
PureProteome™ Lambda Ig Binder Magnetic Beads	2 mL	LSKMAGLM02
PureProteome™ Albumin Magnetic Beads	1 × 10 mL	LSKMAGL10
PureProteome™ Albumin/IgG Depletion Kit (contains magnetic beads, buffer concentrate, and Amicon® Ultra-4 devices)	1	LSKMAGD12
PureProteome™ Human Albumin/ Immunoglobulin Depletion Kit (contains magnetic beads, buffer concentrate, and Amicon® Ultra-2 devices)	1	LSKMAGHDKIT
PureProteome™ Streptavidin Magnetic Beads	2 × 1 mL 1 × 10 mL	LSKMAGT02 LSKMAGT10
PureProteome™ Nickel Magnetic Beads	2 × 1 mL 1 × 10 mL	LSKMAGH02 LSKMAGH10
PureProteome™ NHS FlexiBind Magnetic Beads Kit (contains 0.5 mL magnetic beads, equilibration, wash/coupling, and quench buffers, and Amicon® Ultra-0.5 devices)	1	LSKMAGN01
PureProteome™ NHS FlexiBind Magnetic Beads	4 × 0.5 mL	LSKMAGN04
PureProteome™ Carboxy Flexibind Magnetic Beads		
0.3 µm beads	2 × 1 mL 1 × 10 mL	LSKMAG03CBX02 LSKMAG03CBX10
1.0 µm beads	2 × 1 mL 1 × 10 mL	LSKMAG1CBX02 LSKMAG1CBX10
2.5 µm beads	2 × 1 mL 1 × 10 mL	LSKMAG25CBX02 LSKMAG25CBX10
PureProteome™ Magnetic Stand, 8-well	1	LSKMAGS08
PureProteome™ Magnetic Stand, 15 mL	1	LSKMAGS15

References

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