Protein A Magnetic Beads

Catalog No. MBPP-02001, MBPP-02002

【Introduction】

Protein A Magnetic Beads are nano-superparamagnetic beads covalently coated with protein A. Protein A has high affinity to bind to constant region (Fc) of antibody from multiple species, including human, mouse, rabbit, pig, dog and cat. With a fast magnetic response rate, high protein binding capacity and low non-specific binding, the Protein A Magnetic Beads provide a rapid and efficient method for isolating antibodies from serum, ascites or cell culture media and for immunoprecipitation of the target antigen from cell lysates or tissue extracts. The process can be fully automated.

【Product Specifications】

- **Diameter:** 500nm
- **pH stability:** pH 3-10
- **30min sedimentation rate:** <1%
- **Magnetic response rate:** >30emu/g
- **Solvent:** Phosphate buffered saline, 10% glycerol, Proclin 300
- **Binding capacity:** >40μg human IgG per mg magnetic beads

【Product Content】

Catalog No.	*Conc. (mg/ml)*	*Volume (ml)*	*Amount of Beads (mg)*
MBPP-02001	20	1	20
MBPP-02002	20	5	100

【Protocol】

The following protocol provides general guidelines. The incubation conditions (concentration, time and buffer) and the amount of beads used should be optimized by user for specific application. The protocol is scalable.

A. Notes

1. This protocol includes a general guideline for purification of antibodies from biological samples and immunoprecipitation of the target antigen from cell lysates or tissue extracts.
2. There are two general methods for immunoprecipitation: direct and indirect. With the direct method, protein A magnetic beads bind to an antibody first. The antibody-bound beads can then be used for immunoprecipitation of the target antigen. For indirect target capture, the antibody is incubated with the sample to form the antibody-antigen complex in solution and then beads are added to capture the complex. Indirect target capture is preferred when molecule concentration is low, molecule-target kinetics is slow, affinity is weak, or molecule-target binding requires optimal molecule orientation and true liquid-phase kinetics.
3. Protein A Magnetic Beads are compatible with downstream analyses using mass spectrometry.
4. Wash of Protein A Magnetic beads prior to use is optional.

B. Additional materials recommended

1. Binding/Washing Buffer: Phosphate buffered saline (pH 7.4) or Tris buffered saline (pH 7.4), containing 0.05% Tween-20
2. Elution Buffer: 0.1 M glycine-HCl, pH 3.5
3. Neutralization Buffer: 1M Tris-HCl, pH 8.5
4. Cell Lysis Buffer: NP40 Cell Lysis Buffer
5. Antibody for immunoprecipitation
6. Alternative Elution Buffer: SDS-PAGE reducing sample buffer
7. A magnetic stand or a 96-well magnetic bead automation processor

C. Purification of antibody

This protocol is a general guideline for purification of antibodies from serum, ascites, cell culture media, or other biological samples.

1. Gently mix the magnetic beads thoroughly before use by shaking.
2. Place 50μl of magnetic beads (1mg) into a 1.5ml microcentrifuge tube.

3. Place the tube on a magnetic stand, collect the beads and discard the supernatant.
4. Wash the beads once with Binding/Washing Buffer (500µl) by magnetic separation.
5. Re-suspend the beads in 200µl of Binding/Washing Buffer. Add 50µl of serum or other biological samples. Serum may need dilution prior to the addition to minimize non-specific binding and the dilution fold should be optimized for individual application. Mix the sample and the beads and incubate for 15min at room temperature on a rotator.
6. Collect the beads with a magnet. Wash the antibody-bound beads three times with Binding/Washing Buffer (500µl each time). Transfer the beads to a clean tube at the last wash step to avoid co-elution of protein bound to the tube wall.
7. The antibody can be eluted from beads via incubation with 50µl of Elution Buffer for 5 min at room temperature on a rotator. After the antibody is eluted from beads, apply magnet and transfer the supernatant to a new tube. Add 5µl of Neutralization Buffer to neutralize pH. The purified antibody is ready for use or stored at 4 °C until use.

D. Immunoprecipitation of target antigen

This protocol is a general guideline for immunoprecipitation of target antigen from cell lysates. Recommend using 50µl magnetic beads per 200µl cell lysate containing ~200-500µg of total protein.

1. Cell lysis

1.1. Use PBS wash cells attached to a 60mm culture dish.
1.2. Add 0.5ml of pre-cooled Cell Lysis Buffer to lyse the cells.
1.3. Scrape the cells from the dish. Sonicate the cells for 5 sec four times.Centrifuge the cell lysate at 1000g for 5 min at 4 °C. Obtain the supernatant as the crude cell extract.
1.4. Measure the total protein content of the crude cell extract and use the Cell Lysis Buffer to adjust the total protein concentration to ~1mg/ml.

2. Immunoprecipitation using the direct method

2.1. Transfer 50µl of magnetic beads into a 1.5ml microcentrifuge tube.
2.2. Place the tube on a magnetic stand, collect the beads and discard the supernatant.
2.3. Wash the beads once with Binding/Washing Buffer (500µl each time) by magnetic separation.
2.4. Dilute antibody (1-5µg) in 200µl of Binding/Washing Buffer. Add the antibody solution to the beads and incubate for 10 min at room temperature on a rotator. The amount of antibody used needs to be optimized for specific antibody.

2.5. Collect the beads with a magnet and remove the supernatant. Wash the antibody-bound beads twice with Binding/Washing Buffer (500µ1 each time).
2.6. Add 200µl of the crude cell extract to re-suspend the beads and incubate for 1 hr at 4 °C on a rotator. Incubation time may vary for specific antibody-antigen reaction.
2.7. Collect the beads with a magnet and save the supernatant if desired. Wash the antigen-antibody bound beads twice with Binding/Washing Buffer (500µ1 each time) to remove non-specific binding. Transfer the beads to a clean tube at the last wash step to avoid co-elution of protein bound to the tube wall.

3. Immunoprecipitation using the indirect method

3.1. Add 1-5µg antibody to 200µl of the crude cell extract and incubate for 1 hr at 4 °C on a rotator to form the antibody-antigen complex. Incubation time may vary for specific antibody-antigen reaction.
3.2. Transfer 50µl of magnetic beads into a 1.5ml microcentrifuge tube.
3.3. Place the tube on a magnetic stand, collect the beads and discard the supernatant.
3.4. Wash the beads once with Binding/Washing Buffer (500 µl) by magnetic separation.
3.5. Add the sample containing pre-formed antibody-antigen complex to the beads and incubate for 10 min at room temperature on a rotator.
3.6. Collect the beads with a magnet and save the supernatant if desired. Wash the antigen-antibody bound beads twice with Binding/Washing Buffer (500µ1 each time) to remove non-specific binding. Transfer the beads to a clean tube at the last wash step to avoid co-elution of protein bound to the tube wall.

4. Elution of target antigen

4.1. Non-denaturing elution: The antigen can be eluted from beads via incubation with 50µl of Elution Buffer for 5 min. After the antigen is eluted from beads, apply magnet and transfer the supernatant to a new tube. Add 5µl of Neutralization Buffer to neutralize pH.
4.2. Denaturing Elution: Add 30µl of SDS-PAGE reducing sample buffer to re-suspend the beads, boil the sample for 5 min to denature proteins. Apply magnet, obtain supernatant containing the target antigen for subsequent analysis, e.g. SDS-PAGE gel electrophoresis.

【Storage】

Stored at 2-8°C, 1 year.

【Manufacturer】

Avanbio, ABA-02001, ABA-02002

【Troubleshooting】

A. Low recovery of target protein
   1. Protein may degrade. Add protease inhibitors to cell lysate preparations.
   2. Insufficient magnetic beads. Increase the amount of magnetic beads.
   3. Insufficient target protein is present in sample. Increase the amount of sample; or use a low-molecular-weight-cutoff filter (e.g., molecular weight cutoff of 3,500 Daltons) to reduce the starting sample volume and increase the antibody concentration.
   4. Insufficient incubation time. Incubation time will depend on the concentration of target protein and the affinity of the antibody toward target antigen.
   5. Incorrect elution volume. Elute the samples in volumes 20-60μl. If the level of target antigen is low, a smaller elution volume is recommended. Perform a second elution if desired.

B. Protein does not elute from the beads using low pH buffer
   1. Elution condition is too mild. Increase incubation time with ElutionBuffer; or increase the volume of the Elution Buffer.
   2. Check the pH of the elution buffer. Use an elution buffer with pH3.0-3.5.

C. Multiple non-specific proteins are observed in eluted sample
   1. Nonspecific proteins bind to the beads. Add NaCl (50mM to 350mM) to the Binding/Washing Buffer to increase stringency.
   2. Detergents, such as 0.01–0.1% Tween-20, can be added to the Binding/Washing Buffer to reduce non-specific binding.
   3. Increase the numberof washing steps or the volume of the washing buffer.
   4. Dilute your sample.