INSTRUCTIONS

Protein A Magnetic Beads

Catalog No.: AE02001 AE02002

AvanBio Inc.
239 New Road, Suite A-107
Parsippany, NJ 07054
U.S.A.

Phone: +1 (800) 278-9865
Fax: +1 (855) 378-6845
Email: support@avanbio.com

【Introduction】

EmerTher™ 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 antibodies from multiple species, including human, rabbit, pig, dog and cat. With a fast magnetic response rate, high protein binding capacity and low non-specific binding, the EmerTher™ Protein A Magnetic Beads provide a rapid and efficient method for isolating antibodies from serum, ascites or cell culture media and for immunoprecipitation of a target antigen from cell lysates or tissue extracts. The process can be fully automated.

【Product Specifications】

Diameter：500 nm

pH stability：pH 2-13

Magnetic response rate: >30 emu/g

Solvent: Phosphate buffered saline，10% glycerol，Proclin 300

Binding capacity: 50-100 μg human IgG per mg magnetic beads

【Product Content】

Catalog Number	Conc. (mg/ml)	Volume (ml)	Amount of Beads (mg)
AE02001	20	1	20
AE02002	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 a 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 a target antigen. For indirect target capture, an antibody is incubated with a sample to form an 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. If an antibody or antigen is unstable at room temperature, it is recommended to perform the purification process (binding, washing and elution steps) at 4°C and extend the time for the binding and elution steps as needed.

4. EmerTher™ Protein A Magnetic Beads are compatible with downstream analyses using mass spectrometry.
5. Avoid freezing, drying and centrifugation at a high speed during use and storage of beads. Otherwise, this may decrease the binding capacity of the beads.

B. Additional materials recommended:

1. Binding/Washing Buffer: Phosphate buffered saline (pH 7.4) or Tris buffered saline (pH 7.4), containing 0.02% Triton X-100
2. Elution Solution: 0.1 M glycine-HCl, pH 3.0
3. Neutralization Buffer: 1 M Tris-HCl, pH 8.5
4. Cell Lysis Buffer: 150 mM NaCl，10 mM Tris-HCl (pH 7.4)，1 mM EDTA，1 mM EGTA (pH 8.0)，0.2 mM sodium ortho-vanadate，0.2 mM PMSF，1% Triton X-100，and 0.5% NP-40
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 magnetic beads (e.g. 50 µl, 1 mg) into a 2 ml microcentrifuge tube.
3. Place the tube on a magnetic stand, collect the beads and discard the supernatant.
4. Wash the beads twice with Binding/Washing Buffer (500 µl) by magnetic separation and discard the supernatant.
5. Re-suspend the beads in 200 µl of Binding/Washing Buffer. Add 200 µl 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 at room temperature on a rotator for 15 min.
6. Collect the beads with a magnet and discard the supernatant. Wash the antibody-bound beads three times with Binding/Washing Buffer (1 ml 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 50 µl of Elution Solution at room temperature for 5 min. After the antibody is eluted from beads, apply magnet and transfer the supernatant to a new tube. Add 5 µl of Neutralization Buffer to neutralize pH. The purified antibody is ready for use or stored at 2-8°C until use.

D. Immunoprecipitation of target antigen

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

1. Cell lysis

1.1. Use PBS wash cells attached to a 60 mm culture dish.

1.2. Add 0.5 ml 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 1000 g at 4 °C for 5 min. Obtain the supernatant as a 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 ~1 mg/ml.

2. Immunoprecipitation using the direct method

2.1 Transfer 50 µl of magnetic beads into a 2 ml microcentrifuge tube.

2.2 Place the tube on a magnetic stand, collect the beads and discard the supernatant.

2.3 Wash the beads twice with Binding/Washing Buffer (500 µl each time) by magnetic separation and discard the supernatant.

2.4 Dilute antibody (1-5 µg) in 200 µl of Binding/Washing Buffer. Add the antibody solution to the beads and incubate at room temperature on a rotator for 10 min, so the antibody binds to the beads. 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 (1 ml each time) and discard the supernatant.

2.6 Add 200 µl of the crude cell extract to re-suspend the beads and incubate at 4 °C on a rotator for 1 hr. Incubation time may vary for a 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 (1 ml each time) to remove non-specific binding and discard the supernatant. 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 1-5 µg antibody to 200 µl of the crude cell extract and incubate at 4 °C on a rotator for 1 hr to form an antibody-antigen complex. Incubation conditions may vary for specific antibody-antigen reaction.

3.2 Transfer 50 µl of magnetic beads into a 2 ml microcentrifuge tube.

3.3 Place the tube on a magnetic stand, collect the beads and discard the supernatant.

3.4 Wash the beads twice with Binding/Washing Buffer (1 ml) by magnetic separation and discard the supernatant.

3.5 Add the sample containing pre-formed antibody-antigen complex to the beads and incubate at room temperature on a rotator for 10 min, so the antibody-antigen complex binds to the beads.

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 (1 ml 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 50 µl of Elution Solution for 5 min. After the antigen is eluted from beads, apply magnet and transfer the supernatant to a new tube. Add 5 µl of Neutralization Buffer to neutralize pH.

4.2 Denaturing Elution: Add 30 µl 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.

E. Regeneration

Under non-denaturing elution conditions, the protein A magnetic beads can be reused. Multiple repeated uses may lead to decreased binding of antibodies due to nonspecific adsorption of other proteins.

The beads are stable in aqueous solutions at pH ≤13. Therefore, after the beads are used repeatedly for 5 or more times or a decrease in antibody binding is observed, it is recommended to regenerate the beads using alkali treatment in order to remove non-covalently bound proteins.

1. Add 0.5 ml 0.01 M NaOH solution to 1 mg magnetic beads in a microcentrifuge tube and incubate at room temperature for 15 min.
2. Place the tube on a magnetic stand, collect the beads and discard the supernatant.
3. Wash the beads twice with Binding/Washing Buffer (1 ml) by magnetic separation and discard the supernatant.
4. Re-suspend the beads in Binding/Washing Buffer, the beads are ready for use or stored at 4 °C until use.

【Storage】

Stored at 2-8°C, 9 months

【Troubleshooting】

A. Low recovery of target protein
   1. Protein may degrade. Add protease inhibitors to cell lysate preparations or perform the purification process at 4°C.
   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 an antibody toward target antigen.

5. Incorrect elution volume. Elute the samples in volumes 20-60 µl. 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 Elution Solution; or increase the volume of the Elution Solution.
   2. Check the pH of the elution solution. Use an elution solution with pH 3.0-3.5.

C. Multiple non-specific proteins are observed in eluted sample
   1. Nonspecific proteins bind to the beads. Add NaCl (50 mM to 350 mM) 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 number of wash steps or the volume of the washing buffer.
   4. Dilute your sample.

D. Beads tend to aggregate during binding and are difficult to re-suspend
   1. Insufficient detergent concentration. Increase detergent concentration to 0.1%.
   2. Protein concentration is too high. Reduce protein concentration.

Find out more at AvanBio

This product is for research use only, and is not for use in diagnostic procedures. Please visit our website at www.avanbio.com for MSDS information and more life science research products from AvanBio. For technical support, please email us: support@avanbio.com.