INSTRUCTIONS

MagnaBind™ Protein A and Protein G Beads

21348 21349

1522.2

Number	Description
21348	**MagnaBind Protein A Beads**, 5mL, supplied in PBS with 1mM EDTA and sodium azide, pH 7.4 Binding Capacity: ~0.20mg rabbit IgG/mL of beads
21349	**MagnaBind Protein G Beads**, 5mL, supplied in PBS with 1mM EDTA and sodium azide, pH 7.4 Binding Capacity: ~0.20mg of rabbit IgG/mL of beads

Storage: Upon receipt store products at 4°C. Do not freeze products. Products are shipped at ambient temperature.

Introduction

Thermo Scientific™ MagnaBind™ Beads provide a convenient method for magnetic separation of antibodies, antigens, lectins, enzymes, nucleic acids and cells using affinity binding. To remove the MagnaBind Beads from the suspension, an external magnetic field is used. General characteristics of MagnaBind Beads are listed in Table 1.

MagnaBind Protein A and Protein G Beads are typically used to isolate antibodies from serum and cell culture supernatant and to separate cells of interest from a cell mixture. For antibody purification, the beads are incubated with the antibody solution and then magnetically separated from the supernatant. The antigens and antibodies are then dissociated from the beads using an elution buffer. For cell separation, the monoclonal or polyclonal antibody to the cell surface antigen is incubated with Protein A or Protein G magnetic beads, magnetically separated, and then incubated with the cell suspension.

Table 1. Characteristics of non-derivatized Thermo Scientific MagnaBind Beads.

Composition:	Silanized iron oxide
Magnetization:	25-35EMU/g
Magnetization Type:	Superparamagnetic (no magnetic memory)
Surface Area:	>100m^2/g
Bead Size:	1-4µm diameter
Settling Rate:	4% in 30 minutes
Effective Density:	2.5g/mL
Bead Number:	1×10^8 beads/mg
pH Stability:	Aqueous solution, pH >4.0
Concentration:	5mg/mL

Important Product Information

- Do not freeze, dry or centrifuge MagnaBind Beads. Freezing, drying or centrifuging will cause the beads to aggregate and lose binding activity.
- A low-pH elution may be used for single-use applications; however, using pH < 4 will inactivate the beads and may result in Protein A or Protein G leaching. For multiple use applications, use neutral pH elution conditions such as Gentle Ag/Ab Elution Buffer (Product No. 21027).
- Boiling the beads in SDS-PAGE sample buffer is acceptable for single-use applications, as boiling will cause bead aggregation and loss of binding activity.

Procedure for Antibody Purification

A. Additional Materials Required

- 1.5mL microcentrifuge tubes
- Binding Buffer: IgG (A) Binding Buffer (Product No. 21001 or 21007) or IgG (G) Binding Buffer (Product No. 21011)
- Elution Buffer: IgG Elution Buffer (Product No. 21004 or 21009), or 0.1M glycine, pH 2-3 or Gentle Ag/Ab Elution Buffer (Product No. 21027)
- Neutralization Buffer: 1 ml of high-ionic strength alkaline buffer such as 1M phosphate or 1M Tris; pH 7.5-9
- MagnaBind™ Magnet for a 1.5mL Microcentrifuge Tube (Product No. 21357) or for 6 × 1.5mL Microcentrifuge Tubes (Product No. 21359)
- Thermo Scientific™ Slide-A-Lyzer™ Dialysis Cassette or Dextran Desalting Columns (Product No. 43230) for optional buffer exchange

B. Procedure

Note: Shake beads vigorously before use.

1. Place 500µL of the MagnaBind Beads into a 1.5mL microcentrifuge tube.
2. Add 1mL of Binding Buffer to the tube and invert tube several times to mix. Use the MagnaBind Magnet to separate the beads. Once the supernatant becomes clear, remove and discard the supernatant. Repeat this step three more times.
3. Resuspend the beads in 500µL of Binding Buffer.
4. Add 50µL of serum or cell culture supernatant to the tube and gently invert tube to mix. Incubate tube at room temperature with mixing for 1 hour.
5. Magnetically separate the beads. Once the supernatant becomes clear, remove and discard the supernatant.
6. Add 500µL of Binding Buffer to the tube, mix well, magnetically separate the beads and discard the supernatant. Repeat this wash three times.
7. Add 50µL of Elution Buffer to the tube, mix well and incubate for 5 minutes at room temperature with occasional mixing.
8. Magnetically separate the beads. Once the supernatant becomes clear, remove and reserve the supernatant, which contains the eluted antibody and antigen. To neutralize the low pH, add 2.5µL of Neutralization Buffer for each 50µL of eluate. If desired, perform a buffer exchange by dialysis or desalting.

Procedure for Isolating Lymphocytes

This procedure is for negative or positive cell selection in sterile tissue-culture tubes or flasks. Depending upon antigen availability and the size of the target cell population, positive and negative cell sorting application may require 20-80 magnetic beads per cell. Multiple sorting may also be performed for both positive and negative selection. MagnaBind Beads contain approximately 1×10^8 magnetic beads/mg, and are supplied at 5mg/mL. Because the bead-to-cell ratio is based on the total cell population, the following calculation applies for both positive and negative selection.

Note: Optimal antibody concentrations, incubation times and bead-to-cell ratios must be empirically determined.

A. Sample Calculation for Positive or Negative Selection

This example applies to a system containing 1×10^7 total cells. The target cell population is 30% of the total cells, and a ratio of 50 beads per cell. Beads are supplied at 5mg/mL.

Calculations:

1×10^7 total cells × 50 particles per cell = 5×10^8 MagnaBind Beads required

5mg/mL of beads at 1×10^8 beads/mg equals 5×10^8 beads/mL, therefore, the volume of washed beads required is as follows:

(5×10^8 beads required) × (1mL/5×10^8 beads) = 1.0mL of magnetic beads

B. Selection Procedure

Note: Shake beads vigorously before use.

1. Place ~10 million lymphocytes in a tube in 10mL of RPMI with 5% fetal bovine serum and antibiotics.
2. Wash 1mL of MagnaBind™ Beads three times with 1mL of sterile medium and antibiotics. Between each wash, perform magnetic separation. Shake vigorously after each wash to resuspend the beads. Resuspend in 1mL of sterile medium with antibiotics.
3. Use approximately 5-20µg of monoclonal antibody/1,000,000 target cells. Add the monoclonal antibody to the washed beads. Incubate for 20 minutes at 4°C.
4. Magnetically separate the beads/antibody complex and wash three times with 1mL of sterile medium. Resuspend in 1mL of sterile medium.
5. Add 1mL of washed beads/antibody complex to the 10mL of cells (total bead-to-cell ratio is 50:1) and gently agitate the cell/bead mixture to resuspend the cells.
6. Incubate for 20 minutes at 4°C, agitating the suspension every 10 minutes to promote attachment.
7. Magnetically separate cells for 10 minutes and collect supernatant for a negative selection or save the magnetic pellet for a positive selection.
8. For negative selection, centrifuge and resuspend the cells in medium. For positive selection, continue with the next step.
9. Culture cells after 48 hours of incubation. Cell surface turnover will cause the magnetic beads to detach from the cells. A protease such as chymopapain at ~10 units/10^6 cells for 10 minutes at 37°C also may be used to break the antigen-antibody interaction. MagnaBind Beads are 1-4µm and can be successfully used in flow cytometry equipment, as they will not clog the equipment and are distinguishable from cells.

Additional Information Available on Our Website

- Tech Tip #34: Binding characteristics of Protein A, Protein G, Protein A/G and Protein L
- Tech Tip #43: Protein stability and storage

Related Thermo Scientific Products

32400	**Pierce™ Recombinant Protein A – Peroxidase Conjugated,** 1 mg
26147	**Pierce Crosslink IP Kit**
25200-44	**Precise™ Protein Gels,** see catalog or web site for a complete listing
24590	**GelCode™ Blue Stain Reagent,** 500mL
24612	**Pierce Silver Stain Kit**
24582	**Pierce Zinc Reversible Stain Kit**