TECHNICAL MANUAL

# Magne™ Protein A Beads and Magne™ Protein G Beads for Antibody Purification

Instructions for Use of Products
**G7471, G7472, G7473, G8781, G8782 and G8783**


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TM371

# Magne™ Protein A Beads and Magne™ Protein G Beads for Antibody Purification

**All technical literature is available at: www.promega.com/protocols/**
**Visit the web site to verify that you are using the most current version of this Technical Manual.**
**E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com**



## 1. Description

Magne™ Protein A Beads and Magne™ Protein G Beads[a] are magnetic affinity beads with high specificity and high capacity for binding immunoglobulins from cell culture, ascites and serum samples. These magnetic beads are composed of iron encapsulated by macroporous cellulose, resulting in low non-specific binding. The beads also have excellent magnetic properties that allow rapid and efficient capture using a variety of magnetic stands (see Section 7).

Both beads use a novel attachment chemistry based on the HaloTag® technology to immobilize Protein A or Protein G. The HaloTag® protein is a multi-functional protein tag that forms a covalent bond with its ligand and is used for protein expression and purification, cell imaging, protein immobilization and protein-interaction analysis (see **www.promega.com/halotag/**). The Magne™ Protein A Beads and Magne™ Protein G Beads use recombinant Protein A from Staphylococcus and Protein G from Streptococci, respectively, fused with the HaloTag® protein. The Protein A-HaloTag® (M.Wt 71kDa) or Protein G-HaloTag® (M.Wt 58kDa) fusion protein is covalently attached in an oriented fashion to a magnetic cellulose bead that is activated using a HaloTag® ligand. The bead characteristics are shown in Table 1.

**Table 1. Characteristics of the Magne™ Protein A and Magne™ Protein G Beads.**

| | |
|---|---|
| Composition | Magnetic bead based on macroporous cellulose |
| Chemistry | Oriented and covalent attachment of Protein A or Protein G using HaloTag® technology |
| Particle Size | 30–80µm. |
| Antibody-Binding Capacity | ≥18mg of human IgG/1ml of settled beads |
| Formulation | 20% slurry in 20% ethanol |
| Storage | 2–10°C |

The choice between selecting Magne™ Protein A Beads or Magne™ Protein G Beads depends on the difference in the binding affinities of Protein A or Protein G for antibodies from different species and for different antibody isotypes. See Table 2.

**Advantages of Magne™ Protein A Beads and Magne™ Protein G Beads**

- Simple and easy to use
- High purity and recovery of antibodies
- No expensive instrumentation required
- Processes 1–96 samples in parallel
- Easily handles sample volumes of 20µl to 50ml
- Purification can be automated

Magne™ Protein A Beads and Magne™ Protein G Beads are not recommended for use in immunoprecipitation (IP) or co-IP applications.

Magne™ Protein G Beads are optimized for immunoglobulin purification. Do not use the Magne™ HaloTag® Beads (Cat.# G7281) or HaloLink™ Resin (Cat.# G1912) to bind the Protein G-HaloTag® Fusion Protein (Cat.# G7291), as these products are not optimized for immunoglobulin purification.

**Special Acknowledgement:** The Magne™ Protein G Beads, Protein G HaloTag® Fusion Protein and Magne™ Protein A Beads were developed in collaboration with Kazusa DNA Research Institute (KDRI). We would like to thank KDRI for their technical guidance and early testing.

**Table 2. Binding Affinity of Protein A and Protein G for Different Antibodies and Isotypes**

| Species/Subclass | Protein A | Protein G |
|---|---|---|
| **Monoclonal** | | |
| **Human** | | |
| IgG1 | +++ | +++ |
| IgG2 | +++ | +++ |
| IgG3 | + | +++ |
| IgG4 | +++ | +++ |
| **Mouse** | | |
| IgG1 | + | +++ |
| IgG2A | +++ | +++ |
| IgG2b | ++ | ++ |
| IgG3 | + | ++ |
| **Rat** | | |
| IgG1 | + | + |
| IgG2a | + | +++ |
| IgG2b | + | ++ |
| IgG2c | + | + |
| **Polyclonal** | | |
| Rabbit | +++ | +++ |
| Cow | ++ | +++ |
| Goat | + | +++ |
| Chicken | + | + |
| Human IgG | +++ | +++ |
| Human IgM | + | + |
| Human IgD | + | + |
| Human IgA | + | + |

Key: Strong (+++), Medium (++), Weak (+).
Adapted from Akerström, B. *et al.* (1985) *J. Immunol.* **135(4)**, 2589-92.

## 2. Product Components and Storage Conditions

| PRODUCT | SIZE | CAT.# |
|---|---|---|
| **Magne™ Protein A Beads** | **1.0ml** | **G8781** |
| | **5 × 1.0ml** | **G8782** |
| | **50ml** | **G8783** |
| **Magne™ Protein G Beads** | **1.0ml** | **G7471** |
| | **5 × 1.0ml** | **G7472** |
| | **50ml** | **G7473** |

**Storage Conditions:** Store the Magne™ Protein A and Magne™ Protein G Beads at 2–10°C. Do not freeze. Do not allow beads to dry during storage or use.

Do not reuse the Magne™ Protein A or Magne™ Protein G Beads.

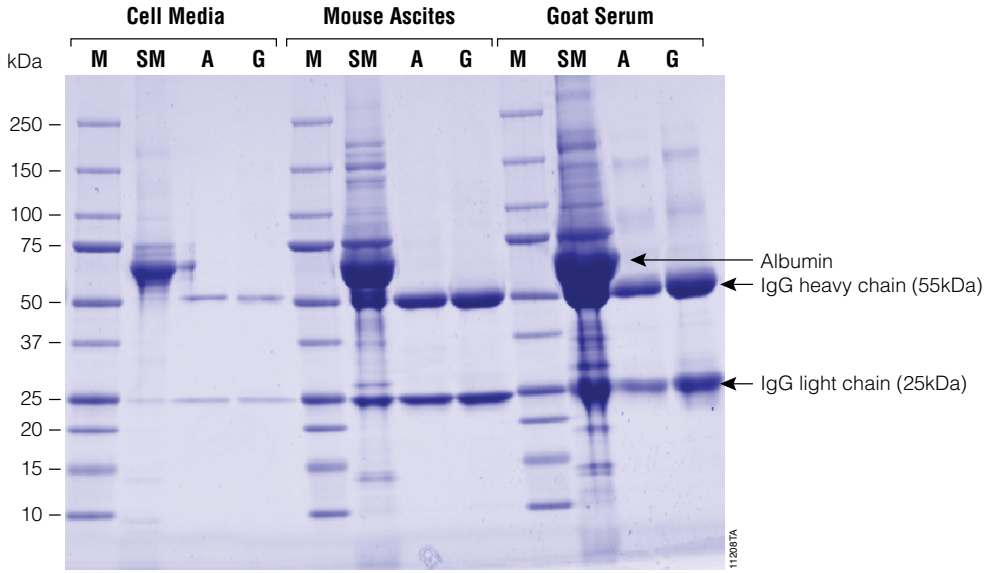


**Figure 1. Antibody purified from various sample types using the Magne™ Protein A and Magne™ Protein G Beads.** Antibody was purified from 50µl of cell culture media (mouse IgG1), mouse ascites (IgG2a) and goat serum using 50µl Magne™ Protein A Beads (A) and Magne™ Protein G Beads (G) as described in this Technical Manual. Samples were analyzed by adding 1µl of starting material (SM) or 5µl of purified sample to SDS buffer, and heating at 80°C for ten minutes before loading onto a 4–20% Tris-glycine gel. The gel was stained with SimplyBlue® Safe Stain.

## 3. Before You Begin

1. Antibody purification using Magne™ Protein A Beads or Magne™ Protein G Beads requires a magnetic stand. The beads can be used with a variety of magnetic stands available from Promega (see Section 7) to purify antibody from 1–96 samples in parallel, with sample volumes ranging from 20µl to 30ml.
2. Be sure that the Magne™ Protein A Beads and Magne™ Protein G Beads remain in suspension during binding and wash steps for maximal antibody yield and purity. We recommend using a tube shaker or end-over-end mixer.
3. Sample incubation times may need to be optimized. Binding may be performed at 4°C; however, longer incubation times may be necessary for efficient antibody capture.
4. Biological samples may be cleared by filtration through a 0.22µm filter or by a 15-minute centrifugation at 14,000 × *g* to remove aggregates and insoluble proteins prior to antibody purification.
5. Prior to antibody purification, cell culture medium can be concentrated using 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. Scale the sample, bind/wash buffer and Magne™ Protein A Bead or Magne™ Protein G Bead volumes proportionally. To minimize sample dilution, a 10X bind/wash buffer may be added to the sample to a final concentration of 1X.
6. Magne™ Protein A Beads and Magne™ Protein G Beads are compatible with several bind/wash buffers, including 25mM sodium acetate buffer (pH 6.0), phosphate-buffered saline (pH 7.4) and Tris-buffered saline (pH 7.5). We recommend an elution buffer of 100mM glycine-HCl (pH 2.7) and a neutralization buffer of 2M Tris buffer (pH 7.5).

**Note:** Purification of different antibody isotypes or antibodies from different species may require protocol optimization for maximal recovery and compatibility with downstream applications.

## 4. Antibody Purification Protocol

Figure 2 shows a schematic of the antibody purification protocol using Magne™ Protein A Beads or Magne™ Protein G Beads. The volume of beads, bind/wash buffer and elution buffer can be scaled proportionally to accommodate different sample volumes and sample types. See Table 3 for guidelines on processing different sample sizes.

MagneSphere® Technology Magnetic Separation Stand.

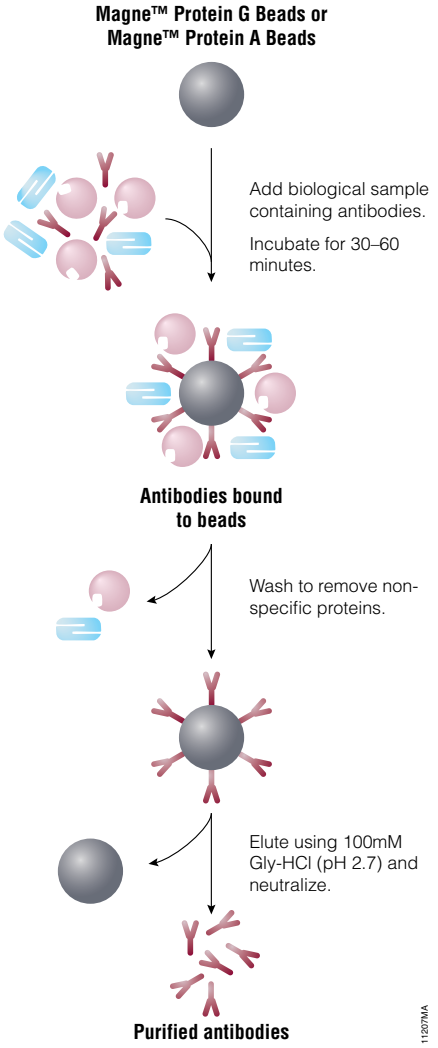


**Figure 2. Schematic of antibody purification using Magne™ Protein A Beads or Magne™ Protein G Beads. Inset:** Magne™ Protein A Beads captured on a magnet.

**Table 3. Guidelines for Antibody Purification.**

| Sample Type | Serum, Ascites, Cell Culture Media | Cell Culture Media | Cell Culture Media | Cell Culture Media | Serum, Ascites, Cell Culture Media |
|---|---|---|---|---|---|
| **Format** | 1.5ml tubes | 1.5ml tubes | 15ml conical | 50ml conical | 96-well plate |
| **Sample Size** | 50µl | 900µl | 9ml | 45ml | 50µl |
| **Bead Slurry** | 50µl | 50µl | 500µl | 2.5ml | 50µl |
| **Amount of Bind/Wash Buffer to Equilibrate Beads** | 500µl | 500µl | 5ml | 25ml | 3 × 150µl |
| **Magnetic Stand (See Section 7)** | MagneSphere® Technology Magnetic Separation Stand (Z5331, Z5332, Z5333, Z5341, Z5342, Z5343) | | PolyATtract® System 1000 Magnetic Separation Stand (Z5410) | | Deep-Well MagnaBot® 96 Magnetic Separation Device (V3031) |
| **Amount of Bind/Wash Buffer to Dilute Sample** | 50µl of 1X bind/wash buffer | 100µl of 10X bind wash buffer | 1ml of 10X bind wash buffer | 5ml of 10X bind wash buffer | 50µl of 1X bind/wash buffer |
| **Bind Time** | 30-60 minutes at room temperature with constant mixing. The beads need to be in suspension for maximal capture. Longer bind times may be necessary at 4°C. | | | | |
| **Wash with Bind/Wash Buffer** | 2 × 500µl, then 1 × 200µl | | 2 × 5ml, then 1 × 1ml | 2 × 25ml, then 1 × 5ml | 3 × 150µl |
| **Elution Buffer** | 2 × 50µl | | 2 × 500µl | 2 × 2.5ml | 2 × 50µl |
| **Neutralization Buffer** | 20µl (10µl for each 50µl elution buffer) | | 200µl (100µl for each 500µl elution buffer) | 1ml (500µl for each 2.5ml elution buffer) | 20µl (10µl for each 50µl elution buffer) |

## 4. Antibody Purification Protocol (continued)

This protocol is for manual antibody purification from 50µl of serum, ascites or cell culture media in a microcentrifuge tube format. See Table 3 for the volume of beads, bind/wash buffer, elution buffer and neutralization buffer to use for different sample sizes.

**Materials to Be Supplied by the User**
(Solution compositions are provided in Section 6.)

- bind/wash buffer
- elution buffer
- neutralization buffer
- magnetic stand
- mixing platform

1. Gently vortex or invert the beads to obtain a uniform suspension. Keep the suspension uniform when aliqotting beads.
2. Add 50µl of bead slurry to a 1.5ml microcentrifuge tube. Place in the magnetic stand for 10 seconds.
3. Remove and discard the storage buffer.
4. Add 500µl of bind/wash buffer. Mix and place in the magnetic stand for 10 seconds. Remove and discard the bind/wash buffer.
5. Combine 50µl of bind/wash buffer and 50µl of sample, then add to the equilibrated beads.
6. Mix sample for 30–60 minutes at room temperature. Make sure the beads remain in suspension by using a tube shaker or end-over-end mixer.
7. Place tube in the magnetic stand for 10 seconds. Remove the supernatant, and save for analysis if desired.
8. Wash beads by adding 500µl of bind/wash buffer and mix for 5 minutes. Place in the magnetic stand for 10 seconds. Remove and discard bind/wash buffer.
9. Repeat Step 8 for a total of two washes.
10. Wash beads by adding 200µl of bind/wash buffer. Mix and place in the magnetic stand for 10 seconds. Remove and discard all bind/wash buffer.
11. Add 50µl of elution buffer [100mM glycine-HCl (pH 2.7)] to the beads.
12. Mix for 5 minutes at room temperature.
13. Place tube in the magnetic stand for 10 seconds. Remove eluted sample, and transfer to a new microcentrifuge tube containing 10µl of neutralization buffer [2M Tris buffer (pH 7.5)]. This is the first elution.
14. Repeat elution Steps 11–13. Eluted samples can be combined.
15. Quantitate the amount of antibody recovered by measuring absorbance at 280nm or using a protein quantitation method such as a Bradford assay. ELISA may be a better quantitative method for samples with low antibody concentration. Check the purity of the antibody using SDS polyacrylamide gel electrophoresis.

## 5. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**
E-mail: **techserv@promega.com**

| Symptoms | Possible Causes and Comments |
|---|---|
| Low antibody recovery | Magnetic beads settled during binding. Increase the mixing speed to maintain beads in suspension during incubations. |
| | Check pH of the elution buffer. Using an elution buffer with a pH greater than 3 will significantly reduce antibody yield. |
| | Remove all bind/wash buffer before elution. The presence of bind/wash buffer will increase the pH of the elution buffer and reduce the amount of antibody eluted. |
| | Protein A and Protein G have different affinities for different antibody isotypes and species (see Table 2). |
| | Elution volume may be decreased to recover antibody at higher concentration. |
| | Low antibody concentration in starting material. 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 (see Section 3). |
| Magnetic beads in the eluted sample | Place eluted sample on the magnetic stand for 10 seconds, and transfer supernatant to a new tube, taking care to avoid the pellet. |
| Beads sticking to tubes in buffer controls | Add 0.005% Tween 20 or IGEPAL® to buffer controls. |
| Magnetic beads in cap after mixing | Centrifuge at 3,000rpm for 1 minute to retrieve beads from the cap. |
| Sample foaming during mixing | Decrease mixing speed. |

## 6. Composition of Buffers and Solutions

**Elution Buffer**
**(100mM glycine-HCl, pH 2.7)**

0.375g glycine

Dissolve in deionized water. Adjust pH to 2.7 with HCl. Bring final volume to 50ml with deionized water.

**Neutralization Buffer**
**(2M Tris buffer, pH 7.5)**

0.472g Trizma base
2.54g Trizma hydrochloride

Dissolve in deionized water. Adjust to pH 7.5. Bring the final volume to 10ml with deionized water.

**Bind/Wash Buffers**

**PBS (pH 7.4)**

137mM NaCl
2.68mM KCl
1.47mM $KH_2PO_4$
8.1mM $Na_2HPO_4$

**25mM Sodium Acetate (pH 6)**

0.17g sodium acetate

Dissolve sodium acetate in 40ml of deionized water. Adjust to pH 6 with HCl. Bring the final volume to 50ml with deionized water.

**Tris-buffered Saline (pH 7.5)**

100mM Tris buffer (pH 7.5)
150mM NaCl

## 7. Related Products

| Product | Size | Cat.# |
|---|---|---|
| MagneSphere® Technology Magnetic Separation Stand (two-position) | 0.5ml | Z5331 |
| | 1.5ml | Z5332 |
| | 12 × 75mm | Z5333 |
| MagneSphere® Technology Magnetic Separation Stand (twelve-position) | 0.5ml | Z5341 |
| | 1.5ml | Z5342 |
| | 12 × 75mm | Z5343 |
| PolyATtract® System 1000 Magnetic Separation Stand | 1 each | Z5410 |
| Deep Well MagnaBot® 96 Magnetic Separation Device | 1 each | V3031 |
| Glycine | 500g | H5071 |
| Tween 20 | 100ml | H5152 |
| Heater Shaker Magnet Instrument (HSM 2.0) | | A2715 |

## 8. Summary of Changes

The following change was made to the 7/15 revision of this document:

1. The document design was updated.

[a]Patent Pending