Protein A Magnetic Beads

1-800-632-7799
info@neb.com
www.neb.com

S1425S 0101502180 21

S1425S

1 ml **Lot: 0101502**

Store at 4°C (Do not freeze) **Exp: 2/18**

Description: Protein A Magnetic Beads are an affinity matrix for the small-scale isolation and purification of immunoglobulins. A truncated form of recombinant Protein A is covalently coupled to a nonporous paramagnetic particle. Protein A exhibits high affinity for subclasses of IgG from many species including human, rabbit and mouse (1). The protein is coupled through a linkage that is stable and leak resistant over a wide pH range. This permits the immunomagnetic purification of IgGs from ascites, serum or cell culture supernatants; the matrix can then be regenerated without loss of binding capacity.

Protein A Magnetic Beads can be used to immunoprecipitate target proteins from crude cell lysates using selected primary antibodies. In addition, specific antibodies can be chemically cross-linked to the Protein A coated surface to create a reusable immunoprecipitation bead, avoiding the co-elution of antibody with target antigen (2,3).

Particles are supplied as a 1 ml suspension in PBS Buffer (pH 7.2), containing 0.1% BSA, 0.05% Tween 20 and 0.02% NaN_3.

Support Matrix: 2 µm nonporous superparamagnetic microparticle.

Binding Capacity: 1 ml of Protein A Magnetic Beads binds > 400 µg of Human IgG.

Protocol

Purification of IgG using Protein A Magnetic Beads:

The following protocol is for the isolation of 10 µg of IgG from approximately 20 µl of human serum.

1. Vortex and thoroughly resuspend Protein A Magnetic Beads.
2. Aliquot 25 µl of bead suspension to a sterile microcentrifuge tube.
3. Add 500 µl of Binding Buffer (0.1 M sodium phosphate, pH 8.0) and vortex to resuspend. Apply magnetic field for 30 seconds to pull beads to the side of the tube (Magnetic Separation Rack [NEB #S1506] holds 6 microcentrifuge tubes). Remove supernatant. Repeat wash.
4. Add 80 µl of Binding Buffer and 15–25 µl of serum to the beads.
5. Mix thoroughly and incubate at 4°C for 30 minutes with agitation.
6. Apply magnetic field and remove supernatant.
7. Wash beads three times as in step 3.

At this point the purified IgG can be eluted from the beads or used directly for immunoprecipitation of target proteins. The purified IgG can also be cross-linked to the Protein A Magnetic Beads to create a reusable immunoprecipitation bead which prevents the co-elution of antibody with target protein (2,3).

IgG Elution:

1. Add 50 µl of 0.2 M glycine (pH 2.5) to the bead pellet, vortex and incubate for 5 minutes at 4°C with agitation.
2. Apply magnetic field and transfer eluted IgG to clean microcentrifuge tube.
3. Add an additional 50 µl of 0.2 M glycine to the beads and repeat elution step. Pool elution supernatants and neutralize by adding 20 µl of 1 M Tris-HCl (pH 9.0). **Store at 4°C**.

(See other side)

CERTIFICATE OF ANALYSIS

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(See other side)

CERTIFICATE OF ANALYSIS

Immunoprecipitation using Protein A Magnetic Beads: Use 25 µl of Protein A Magnetic Beads per 200 µl of crude cell lysate containing 200–500 µg of total protein in a standard immunoprecipitation protocol. It is important to increase the volume of beads proportionately for larger cell lysate volumes.

Cell Lysis:

1. Rinse a 60 mm culture dish of confluent cells with PBS.
2. Lyse the cells with 0.5 ml cold Immunoprecipitation 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, 0.5% NP-40).
3. Maintain constant agitation for 30 minutes at 4°C.
4. Scrape the cells from the dish. Sonicate on ice for 5 seconds; repeat 4 times. Centrifuge for 5 minutes at 4°C. The supernatant is the crude cell lysate. Assay for total protein then adjust concentration to approximately 1 mg/ml with Immunoprecipitation Buffer.

Immunoprecipitation:

1. This step pre-clears crude cell extract of proteins which can bind non-specifically to the beads. In a 1.5 ml microcentrifuge tube, add 25 µl Protein A Magnetic Beads to 200 µl of crude cell extract. Gently vortex and incubate at 4°C for 1 hour. Apply magnetic field for 30 seconds to pull beads to the side of the tube. Pipette supernatant to a clean 1.5 ml microcentrifuge tube. Discard beads.
2. Add 1–5 µg of antibody to crude cell lysate vortex and incubate at 4°C for 1 hour. (If monoclonal antibodies are used, add 5 µg rabbit anti-mouse IgG antibody. Vortex and incubate an additional 30 minutes at 4°C).*
3. Add 25 µl of Protein A Magnetic Beads suspension. Gently vortex and incubate with agitation for 1 hour at 4°C.
4. Apply magnetic field to pull beads to the side of the tube. Carefully pipette to remove supernatant.
5. Wash with 500 µl of Immunoprecipitation Buffer by gentle vortex. Apply magnetic field then remove supernatant and discard. Repeat wash 2 times.
6. Resuspend bead pellet in 30 µl of 3X SDS Sample Loading Buffer (187.5 mM Tris-HCl (pH 6.8), 6% (w/v) SDS, 30% glycerol, 150 mM DTT, 0.03% (w/v) bromophenol blue, 2% β-mercaptoethanol).
7. Incubate sample at 70°C for 5 minutes.
8. Apply magnetic field to sample then load supernatant on SDS-PAGE gel and electrophorese.

*Alternatively, Protein G Magnetic Beads (NEB #S1430S) can be used for immunoprecipitations with monoclonal antibodies.

References:

1. Harlow, E. and Lane, D. (1988). Bacterial Cell Wall Proteins that Bind Antibodies. *Antibodies*: *A Laboratory Manual,* (pp. 615–619). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
2. Schneider, C., Newman, R.A. et al. (1982) *J. Biol. Chem*. 257, 10766.
3. Sisson, T.H. and Castor, C.W. (1990) *Immunol. Methods*, 127, 215.

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