Goat Anti-Rabbit IgG **Magnetic Beads**



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



20 mg

Lot: 0061211 Exp: 11/14

Store at 4°C (Do not freeze)

Description: An affinity matrix for the small-scale immunomagnetic separation and purification of rabbit IgG's. Goat Anti-Rabbit IgG is covalently coupled to a nonporous paramagnetic particle. This secondary antibody binds the heavy chain of all rabbit IoG subclasses and is suitable for immunoassays that employ a rabbit IgG primary polyclonal antibody. Cell separations and sorting can be accomplished using a rabbit IgG antibody to defined cell surface antigens.

Supplied as a 1 ml suspension in PBS Buffer (pH 7.4), containing 0.05% Tween 20, 0.1% BSA and 0.05% NaN₃.

Specifications:

Particle Concentration: 3.65 x 10¹⁰ particles/ml

Support Matrix: 1 µm nonporous paramagnetic microparticle.

Binding Capacity: 1 mg of Goat Anti-Rabbit IgG Magnetic Beads will bind 5 µg of rat lgG.

Note: The amount of antibody required for optimal coating of the beads will vary with the PAb affinity and the antigen density on the cell surface. Optimal experimental conditions should be determined by titration on an individual basis.

Protocol

Cell separation by direct method: Thoroughly suspend goat-anti rabbit IgG magnetic particles by vortexing followed by end over end mixing for at least 1 hour at 4°C.

- 1. Aliquot 10 µl of bead solution to clean microcentrifuge tube and wash 3X with 1 ml of cold 1X PBS (pH 7.5) or sterile media containing antibiotics.
- 2. Add 5–10 µg of antibody to 20 µl 1X PBS and add to washed magnetic beads. Incubate at 4°C with agitation for at least 1 hour.
- 3. Place tube in NEB Magnetic Separation Rack to pull beads to the side of the tube and decant supernatant being careful not to disturb bead pellet.
- 4. Wash 4X as in step 2. Suspend beads in 100 ul of storage buffer appropriate for the primary antibody.

- 5. Incubate primary antibody coated beads with heterogeneous cell suspension for 30 minutes at 4°C. Gently agitate the incubating suspension every 10 minutes. Use a magnetic bead to target cell ratio of greater than or equal to 5 magnetic beads per target cell. Incubation volume should be at least 1 ml for $> 1 \times 10^7$ cells to reduce non-specific binding and clumping. Addition of 5% Fetal Bovine Serum to media and buffers may also serve to reduce non-specific binding.
- 6. Magnetically separate beads to the side of the tube for at least 10 minutes. Save the supernatant for a negative selection or save the magnetic pellet for a positive selection.
- 7. Cultured cells may detach from magnetic beads by incubating cells for up to 48 hours. Proteases such as chymopapain and trypsin can be used in some instances to release cells or interrupt antigen-antibody interaction.

CERTIFICATE OF ANALYSIS

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S1432S

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