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Protocol

TrueBlot® Anti-Rabbit IgG Magnetic Beads

Rockland's TrueBlot® Anti-Rabbit IgG Magnetic Beads are uniform, non-aggregating, super-paramagnetic beads coupled with a biomolecule, such as goat anti-rabbit IgG. These beads are specifically designed, tested and quality controlled for isolation and purification of rabbit IgG and its target proteins, and for immunoprecipitation methods. This antibody binds the heavy chain of rabbit IgG and is suitable for immunoassays that utilize a rabbit IgG primary polyclonal antibody. Cell separation and sorting can be achieved using a rabbit IgG antibody to defined cell surface antigens. The beads have a large surface area with high capture efficiencies.

Compatible Products

Product	Size	Item No.
TrueBlot® Anti-Rabbit IgG Magnetic Beads	2 mL	00-1800-20
TrueBlot® Anti-Rabbit IgG Magnetic Beads	5 mL	00-1800-50

Note: The anti-rabbit IgG magnetic beads should be stored in the refrigerator (2–8°C). The reagent must be allowed to reach room temperature (20–25°C) before use and may be used until the expiration date (one year from date of opening). Do not freeze, dry, or centrifuge the beads as it may result in the loss of binding activity and/or aggregation.

Product Characteristics

Bead Mean Diameter	5 µm
Bead Concentration	5 mg/mL
Binding Capacity	≥ 200 µg rabbit IgG/mg of beads

Reagents Required

Product
Binding/Wash Buffer: TBS 0.05% TWEEN® 20 or PBS 0.05% TWEEN 20
IP Lysis Buffer
Elution Buffer: 0.1 M Glycine, pH 2.0
Neutralization Buffer: 1 M Tris HCl, pH 8.0
Primary Antibodies
Antigen Sample

Procedure for Antibody Isolation

1. Add 100 µL (0.5 mg) of beads to 1 mL of binding buffer in each tube to wash beads.
2. Magnetically separate the beads using a magnetic separator for 2 minutes or when the supernatant is clear.
3. Remove the supernatant and wash once more by adding 1 mL of binding buffer.
4. Magnetically separate the beads using a magnetic separator for 2 minutes or when the supernatant is clear and remove the supernatant.
5. Re-suspend beads by adding 450 µL of binding buffer.
6. Add 50 µL of serum or cell culture supernatant to the beads.
   Note: Sample volume can be modified according to user preference. If the sample volume is < 500 µL, dilute it to a final volume of 500 µL with binding/wash buffer.
7. Gently mix using vortex or rotator for 30 minutes.
8. Magnetically separate using a magnetic separator for 2 minutes or when the supernatant is clear.
9. Remove supernatant and wash with 0.5 mL binding/wash buffer to remove unbound proteins.
10. Magnetically separate using a magnetic separator for 2 minutes or when the supernatant is clear.
11. Remove supernatant and wash with 0.5 mL binding/wash buffer.
12. Add 100 µL of elution buffer to beads and mix well.
13. Incubate at room temperature for 10 minutes with occasional gentle mixing or vortexing.
14. Separate for 2 minutes and remove the eluent to a new tube containing 15 µL of neutralization buffer.

Procedure for Immunoprecipitation

Immunoprecipitation (IP) is performed by either adding the primary rabbit antibody to goat anti-rabbit IgG magnetic beads followed by adding the sample antigen, or by first forming an antigen-antibody complex and then incubating the complex with goat anti-rabbit IgG magnetic beads. Bound antigen-antibody complex is then eluted using different elution methods.

The amount of antibody captured depends on the concentration of antibody and anti-rabbit IgG magnetic beads in the starting sample. For standard IP, use PBS or TBS for antibody binding and washing steps. However, these may be substituted by other buffers of choice, such as phosphate buffers, lysis buffer (e.g. RIPA, NP40), or HEPES. The recommended elution buffer may also be substituted by alternative low pH, high pH or high salt buffers, depending on the target protein and downstream application.

Note: The amount of sample needed and the incubation time are dependent upon each specific antibody-antigen system and may require optimization by the user for maximum yield. The following protocol is for 2–10 µg of affinity-purified antibody and can be scaled up as needed.

1. Combine cell lysate containing the antigen (typically 100–1000 µL) with 2–10 µg of IP antibody per sample in a microcentrifuge tube.
   Note: The suggested amount of total protein per IP reaction is 500–1000 µg, as determined by the BCA Protein Assay.
2. Dilute the antibody/lysate solution to 500 µL with IP lysis/wash buffer.
3. Incubate for 1 hour at room temperature or overnight at 4°C to form the immune complex (the optimal incubation time can be determined in a preliminary experiment).

4. Place 100 µL (0.5 mg) of goat anti-rabbit IgG magnetic beads into a 1.5 mL microcentrifuge tube.
5. Add 1 mL of IP lysis/wash buffer to the beads and gently vortex to mix.
6. Place the tube into a magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant.
7. Add 1 mL of IP lysis/wash buffer to the tube. Invert the tube several times or gently vortex to mix for 1 minute. Collect beads with magnetic separator. Remove and discard the supernatant.
8. Add the antigen-antibody (Ag-Ab) complex to the tube containing pre-washed magnetic beads and incubate at room temperature for 1 hour with mixing.
9. Collect the beads with a magnetic separator (TMS-15-50, TMS-06, or TMS-32), remove the unbound sample and save for further analysis if desired.
10. Add 50 µL of IP lysis/wash buffer to the tube and gently mix. Collect the beads and discard the supernatant. Repeat this wash twice.
11. Wash 100 µL (0.5 mg) of goat anti-rabbit IgG magnetic beads as described above. Re-suspend beads by adding 450 L of binding/wash buffer.
12. Add 2–10 µg (50 µL) of IP antibody to the bead slurry.
13. Incubate the antibody-bead mixture for 1 hour at room temperature or 2–4 hours at 4°C by gently mixing the mixture on a rotator.
14. Place the tube into a magnetic stand to collect the antibody-bead mixture against the side of the tube. Remove and discard the supernatant.
15. Add 1 mL of IP lysis/wash buffer to the tube. Invert the tube several times or gently vortex to mix for 1 minute. Collect beads with magnetic separator. Remove and discard the supernatant. Repeat this wash step one more time.
16. After washing the beads and antibody mixture, add cell lysate containing the antigen (100–500 µL) and gently re-suspend the lysate-bead/antibody conjugate mixture.
17. Incubate the lysate-bead/antibody conjugate complex for 1 hour at room temperature or overnight at 4°C under rotary agitation (the optimal incubation time can be determined in a preliminary experiment).
18. Collect the beads with a magnetic separator, remove the unbound sample and save for further analysis if desired.
19. Add 500 µL of IP lysis/wash buffer to the tube and gently mix. Collect the beads and discard the supernatant. Repeat this wash twice

Low-pH Elution

1. Add 100 µL of elution buffer to the tube.
2. Incubate the tube at room temperature with mixing for 10 minutes.
3. Magnetically separate the beads and save the supernatant (supernatant contains target antigen).
4. To neutralize the low pH, add 15 µL of neutralization buffer for each 100 µL of eluate.

Alternative Elution

1. Add 100 µL of sample buffer to the tube and heat the samples at 96–100ºC in a heating block for 10 minutes.

2. Magnetically separate the beads and save the supernatant (supernatant contains target antigen).
   Note: If elution under reducing conditions is desired, add DTT (to a final concentration of 50mM) to the 1X Sample Buffer

Notes

- Ensure that reagent bottle caps are replaced tightly after each use to prevent drying of reagents.
- Use a mixer that provides tilting and rotation of the tubes to ensure that the beads do not settle in the tube.
- Avoid air bubbles (foaming) during pipetting.
- Use recommended volume of beads and carefully follow the recommended volumes and incubation times.
- Mistakes in handling the test can also cause errors. Possible sources for such errors can be: Inadequate storage conditions of the test reagents, incorrect pipetting sequence or inaccurate volumes of the reagents, insufficient incubation times, and/or insufficient magnetic separation times.
