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Protocol

TrueBlot® Biotin Magnetic Beads

Rockland's TrueBlot® Magnetic Beads are uniform, non-aggregating, super-paramagnetic beads consisting of a ferric oxide core functionalized with various silane groups. The super-paramagnetic nanoparticles are coupled with a biomolecule, such as Biotin, and are specifically designed, tested and quality controlled for magnetic separation, isolation and purification of avidin and streptavidin-labeled components using manual or automatic platforms. Biotin magnetic beads are stable, pre-blocked beads with high binding capacity that provide rapid and efficient biomolecule purification from complex samples.

TrueBlot® Biotin magnetic beads can be used in immunoassays or molecular diagnostics when avidin, streptavidin is bound to proteins or oligonucleotides. Biotin magnetic beads can also be used in nucleic acid isolation, protein purification, and cell separations. Biotin magnetic particles are incubated with the avidin or streptavidin labeled solution and then separated by magnets. After the unbound particulates are washed from the particles, the bound avidin is eluted from the particles using the elution buffer. The particles are then magnetically separated from the eluted solution, which is removed manually.

Compatible Products

Product	Size	Item No.
TrueBlot® Biotin Magnetic Beads	2 mL	B000-18-2
TrueBlot® Biotin Magnetic Beads	5 mL	B000-18-5

Note: The Biotin Magnetic Beads should be stored in the refrigerator (2-8°C). The beads must be allowed to reach room temperature (20-25°C) before use. Do not freeze, dry, or centrifuge the particles as it may result in loss of binding activity and/or aggregation. Do not use after the printed expiration date.

Reagents Required

Product	Item No.
Magnetic Separators	TMS-15-50/ TMS-06 / TMS-32
Mixer for tilting and rotation of tubes	
Binding/wash buffer: TBS-0.05% Tween 20 or PBS-0.05% Tween 20	
Elution Buffer: 0.1 M Glycine pH 2.0	
Neutralization Buffer: 1M Tris-HCl pH 8.0	
1.5 mL or 2.0 mL Eppendorf or microcentrifuge vials	
Timer	
Distilled or deionized water	

Product Characteristics

Bead Mean Diameter	5 µm
Bead Concentration	5 mg/mL
Binding Capacity	≥ 30µg streptavidin/mg of beads

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.

Notes

- Do not centrifuge, dry or freeze the magnetic beads, as this can cause the beads to aggregate and/or lose binding capability.
- Prior to use, ensure that the product has not expired by verifying the expiration date on the label.
- 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.
- Carefully follow the recommended volumes and incubation times.

- Mistakes in handling the test can cause errors. Possible sources for such errors are 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.
