INSTRUCTIONS

MagnaBind™ Carboxyl Derivatized Beads

21353

0726.5

Number	Description
21353	**MagnaBind Carboxyl Derivatized Beads**, 5mL, supplied in water Loading: ~4.8µmol carboxyl/mL (240µmol/g)

Storage: Upon receipt store product at 4°C. Do not freeze product. Product is shipped at ambient temperature.

Introduction

Magnetic separation is a convenient method for isolating antibodies, antigens, lectins, enzymes, nucleic acids and cells using affinity binding, while retaining biological activity. To remove the Thermo Scientific MagnaBind Beads from the suspension, an external magnetic field is used.

MagnaBind Beads can be used for affinity chromatographic procedures to purify specific molecules from a complex mixture. They offer rapid separations, high recovery and high specificity. MagnaBind Beads make it possible to isolate single populations of cells, specific proteins and nucleic acids. MagnaBind Carboxyl Derivatized Beads are supplied as an aqueous suspension of magnetic iron oxide beads coated with carboxyl groups for covalent coupling of molecules using EDC, a zero-length crosslinker that is amine- and carboxyl-reactive. Additionally, carboxyl derivatized beads have been used to purify plasmid DNA from cells.[1] General characteristics of MagnaBind Beads are listed in Table 1.

Table 1. Characteristics of Thermo Scientific MagnaBind Carboxyl Derivatized Beads.

Composition:	Silanized iron oxide
Magnetization:	25-35EMU/g
Type of Magnetization:	Superparamagnetic (no magnetic memory)
Surface Area:	>100m^2/g
Bead Size:	1-4µm diameter
Settling Rate:	4% in 30 minutes
Effective Density:	2.5g/mL
Number of Beads:	1×10^8 beads/mg
pH Stability:	Aqueous solution, above pH 4.0
Concentration:	~20mg/mL

Important Product Information

- Do not dry, freeze or centrifuge MagnaBind Beads. Freezing, drying or centrifuging will cause the beads to aggregate and lose activity.
- If MagnaBind Beads are used to recover a molecule by affinity purification, low-pH elution (pH<4) may be used for single-use applications; however, using pH<4 will inactivate the beads and may result in leaching. For multiple use applications, use neutral pH elution conditions such as Gentle Ag/Ab Elution Buffer (Product No. 21027).
- Boiling the beads in SDS-PAGE sample buffer is acceptable for single-use applications, as boiling will cause bead aggregation and loss of activity.
- For microbe-free preparations, the MagnaBind Beads may be washed with antibiotic medium or gamma-irradiated.

Example Protocol I: Purification of Plasmid DNA using Carboxylated Beads

The following method is derived from Hawkins, *et al.* and outlines the use of MagnaBind Carboxyl Derivatized Beads to purify and isolate plasmid DNA. This procedure uses polyethylene glycol and a high concentration of salt to bind DNA to the carboxylated beads. After the DNA-bead complex is thoroughly washed, the DNA is eluted with water.

Additional Materials Required

- Overnight culture containing plasmid clone
- Solution I: 50mM glucose, 25mM Tris•HCl, pH 8, 10mM EDTA, 100µg/mL RNase
- Solution II: 0.2N NaOH, 1% SDS
- Solution III: 3M KOAc
- 0.5M EDTA, pH 7.2
- Binding Buffer: 20% PEG 8000, 2.5M NaCl
- Wash Buffer I: 5M NaCl
- Wash Buffer II: 25mM Tris acetate, pH 7.8, 100mM KOAc, 10mM Mg_2OAc, 1mM DTT
- MagnaBind Magnet (see Related Thermo Scientific Products section)

Procedure

1. Place 1mL of overnight culture containing the plasmid clone into a microcentrifuge tube.
2. Centrifuge on high for 2 minutes to pellet the cells. Decant the supernatant and resuspend the cell pellet in 30µL of Solution I.
3. Add 60µL of Solution II and mix by shaking. Incubate at room temperature for 5 minutes.
4. Add 45µL of Solution III, mix by shaking and incubate on ice with shaking for 10 minutes.
5. Centrifuge for 10 minutes and remove 100µL of the supernatant to a new microcentrifuge tube.
6. Shake MagnaBind Beads vigorously. Wash 10µL (20mg/mL) of the MagnaBind Carboxyl Derivatized Beads three times in 0.5M EDTA, pH 7.2.
7. Resuspend beads in 10µL of 0.5M EDTA, pH 7.2 and add to the cleared lysate.
8. Add 100µL of Binding Buffer and mix.
9. Incubate at room temperature for 5 minutes.
10. Wash beads two times with Wash Buffer I, followed by one wash with Wash Buffer II. It is not necessary to resuspend the beads after each of these washes.
11. Resuspend beads in 50µL of ultrapure water and incubate at room temperature for 1 minute.
12. Magnetically separate beads from the DNA. Perform magnetic separation perpendicular to gravity. Transfer DNA (i.e., supernatant) to a new tube.

Example Protocol II: Crosslinking Proteins or Peptides to Carboxylated Beads

Additional Materials Required

- Phosphate buffered saline (PBS): 100mM phosphate, 150mM NaCl, pH 7.2 (Thermo Scientific BupH Phosphate Buffered Saline Packs, Product No. 28372)
- EDC [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride] (Product No. 22980)
- Conjugation Buffer: 0.1M MES (*N*-morpholinoethane sulfonic acid), 0.9% NaCl; pH 4.7. (BupH™ MES Buffered Saline Packs, Product No. 28390)
- MagnaBind Magnet (see Related Thermo Scientific Products section)

Procedure

Note: Shake beads vigorously before use.

1. Wash 1mL of MagnaBind Beads three times with 1mL of PBS. Gently agitate after each wash. Magnetically separate and aspirate beads after each wash. Perform magnetic separation perpendicular to gravity
2. Dissolve the primary amine-containing peptide or protein in conjugation buffer at a concentration of 5-10mg/mL.

   Note: Coupling efficiency can be determined by measuring the absorbance of the protein/peptide solution at 280nm before and after crosslinking.
3. Add 1mL of the protein/peptide to the washed MagnaBind Beads and gently agitate.
4. Dissolve 10mg of EDC in 1mL of Conjugation Buffer immediately before use.
5. Add 0.1mL of the EDC solution to the beads-peptide/protein mixture and gently agitate. Incubate reaction for 30 minutes at room temperature.
6. Use a MagnaBind Magnet Separation Unit to separate protein/peptide coupled to the beads from the solution.
7. Aspirate supernatant from the coupled beads. Wash beads three times with 1mL PBS.
8. Measure the absorbance of the supernatant at 280nm and subtract value from starting amount to determine coupling efficiency. Alternatively, measure the amount of immobilized protein according to Tech Tip #9: Quantitate immobilized protein, from the Technical Resources section of our web site.

Related Thermo Scientific Products

21358	**MagnaBind Magnet for 96-Well Separator**, 1 each
21357	**MagnaBind Magnet for a single 1.5mL Microcentrifuge Tube**, 1 each
21359	**MagnaBind Magnet for six Microcentrifuge Tubes**, 1 each
22980	**EDC**, 5g
28390	**BupH MES Buffered Saline Packs,** 10 packs
28372	**BupH Phosphate Buffered Saline Packs,** 40 packs
21030	**Gentle Ag/Ab Binding and Elution Buffer System**, 100mL each
21027	**Gentle Ag/Ab Binding and Elution Buffer System**, 500mL
21348	**MagnaBind Protein A Beads**, 5mL
21349	**MagnaBind Protein G Beads**, 5mL

Cited Reference

1. Hawkins, T.L., *et al.* (1994). DNA purification and isolation using a solid-phase. *Nucl Acid Res* **22(21):**4543-4.