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A Geno Technology, Inc. (USA) brand name

Carboxyl Magnetic Beads

(Cat. # 786-908, 786-909)

INTRODUCTION

G-Biosciences' Carboxyl Magnetic Beads are magnetic beads with surface functional group -COOH. The magnetic bead consists of a single-crystal Fe_3O_4 sphere core and dextran coating layer. Through chemical modification of dextran, the carboxyl groups (-COOH) are joined to the magnetic beads through a short hydrophilic linker. The hydrophilic surface ensures the magnetic beads excellent dispersion ability and easy handling property in a wide variety of buffers.

Molecules can be coupled to the free amine by numerous amine-reactive methods; however the use of the carbodiimide EDC allows coupling of free carboxyl groups. The resulting amide bond is highly stable and greatly reduces the chance of leaching of the affinity tag.

Carbodiimide activation of carboxyl groups produces a very labile intermediate that hydrolyzes quickly, meaning the ligand needs to be added rapidly. Alternatively, a two step protocol using N-hydroxysuccinimide (NHS) can be employed to produce a less labile intermediate that reacts over a longer time period. Protocols for both methods are below.

ITEMS SUPPLIED

Cat. #	Description	Size
786-908	Carboxyl Magnetic Beads	1ml
786-909	Carboxyl Magnetic Beads	5ml

STORAGE CONDITIONS

The beads are shipped at ambient temperature. Upon arrival, store the beads at 4°C. If stored and handled correctly the beads have a 1 year shelf life.

SPECIFICATIONS

Fe_3O_4 beads coated with dextran of an average 1µm in diameter. Carboxyl group, more than 2mM, is coupled covalently to dextran. Carboxyl Magnetic Beads are supplied in phosphate buffered saline, pH 7.4.

PRECAUTIONS

- Do not freeze the magnetic beads
- Do not store near magnetic sources

ADDITIONAL ITEMS REQUIRED

- Coupling Buffer (0.1M MES Buffer, 0.9% NaCl)
  NOTE: *For coupling reactions using EDC avoid the use of buffers containing free amines or phosphates as these will interfere with coupling efficiency. Tris, acetate and glycine buffers all readily react with EDC or the coupling intermediate. Thiol containing buffers should also be avoided as these irreversibly bind EDC and inhibit coupling.*
- EDC; 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (Cat. # BC25-1, BC25-5)
- Sulfo-NHS or NHS
- Quenching Buffer (50mM Tris, pH 8.0 or 5-10mM Hydroxylamine)
- PBS
- Magnetic Stand or magnet

PROTOCOL

Preparation of Carboxyl Magnetic Beads

1. Resuspend the Carboxyl Magnetic Beads thoroughly by pipetting or vortexing the vial for 15 seconds.
2. Transfer adequate amount (100µl) of Carboxyl Magnetic Beads into a clean tube.
3. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall.
4. Discard the supernatant by aspiration with a pipette.
5. Remove the tube from the magnetic stand.
6. Add 200µl Coupling Buffer and resuspend the beads by pipetting.
7. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall.
8. Discard the supernatant, and then remove the tube from the magnetic stand.
9. Repeat steps 6-8 twice.

Two Step Activation with NHS

1. Prepare 50mg/ml EDC solution in MES Buffer and 50mg/ml sulfo-NHS or NHS solution in MES Buffer respectively.
   NOTE*: Both EDC solution and NHS solutions should be prepared freshly, protected from light, and kept on ice before use.*
2. Add 60µl MES Buffer, 20µl EDC solution and 20µl NHS solution to the prepared beads, and resuspend the beads by pipetting.
3. Incubate for 15 minutes at room temperature with rocking.
4. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall.
5. Discard the supernatant, and then remove the tube from the magnetic stand.
6. Add 50µl MES Buffer with 0.1-3mg/ml antibody or ligand and resuspend the beads by pipetting.

7. Incubate with tilt rotation at room temperature for 30 minutes or at 4°C overnight.
8. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall.
9. Discard (or collect, if desired) the supernatant as unbound substances, and then remove the tube from the magnetic stand.
10. Add 100µl MES Buffer and resuspend the beads by pipetting.
11. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall.
12. Discard the supernatant, and then remove the tube from the magnetic stand
13. Go to the Quenching Protocol to block unreacted sites.

One Step Activation

1. Prepare 50mg/ml EDC solution in MES Buffer.
   NOTE*: The EDC solution should be prepared freshly, protected from light, and kept on ice before use.*
2. Add 50µl MES Buffer with 0.1-3mg/ml antibody or ligand and resuspend the beads by pipetting.
3. Add 60µl MES Buffer and 20µl EDC solution to the prepared beads, and resuspend the beads by pipetting.
4. Incubate for 30 minutes at room temperature with rocking.
5. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall.
6. Discard (or collect, if desired) the supernatant as unbound substances, and then remove the tube from the magnetic stand.
7. Add 100µl MES Buffer and resuspend the beads by pipetting.
8. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall.
9. Discard the supernatant, and then remove the tube from the magnetic stand
10. Repeat steps 8-9 twice.

Quenching Protocol

1. Add 500µl Quenching Buffer and resuspend the beads by pipetting.
2. Incubate with tilt rotation for 30 minutes at room temperature.
3. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall.
4. Discard the supernatant, and then remove the tube from the magnetic stand.
5. Add 500µL Quenching Buffer and resuspend the beads by pipetting.
6. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall.
7. Discard the supernatant, and then remove the tube from the magnetic stand.
8. Add 500µL PBS, pH 7.4 (or the buffer preferred) and resuspend the beads by pipetting.
9. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall.
10. Discard the supernatant, and then remove the tube from the magnetic stand.
11. Repeat steps 8-10 twice.
12. Add 100µL PBS, pH 7.4 (or the buffer preferred) and resuspend the beads by pipetting. Store the beads at 4°C.

APPENDIX 1: IMMUNOPRECIPITATION METHOD

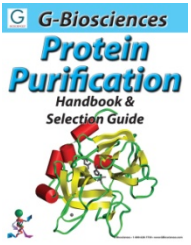
1. Add at least 100µl cell lysate sample containing target antigen to the tube from step 17 and vortex for 10 seconds.
2. Incubate for 30 minutes at room temperature or 4°C overnight with gentle rocking or shaking.
3. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall.
4. Discard the supernatant, and then remove the tube from the magnetic stand.
5. Add 200µl Washing Buffer and resuspend the beads by pipetting.
6. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall.
7. Discard the supernatant, and then remove the tube from the magnetic stand.
8. Repeat steps 5-7 two more times to remove unbound antigen.
9. For SDS-PAGE and Western blot analysis, add appropriate volume of SDS-PAGE Loading Buffer and heat the beads at 95°C for 5 minutes. Load the bead/ loading buffer mix directly onto an SDS-PAGE gel and proceed with electrophoresis and blotting as normal.

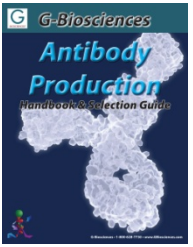
ELUTION OF ANTIBODY & ANTIGEN

1. Add 20µl Elution Buffer to the bead:antibody:antigen mix and resuspend by pipetting.
2. Incubate with tilt rotation for 2 minutes at room temperature.
3. Place the tube on the magnet stand for 30-60 seconds.
4. Collect the supernatant to a clean tube, and then adjust the pH by adding 2µl Neutralization Buffer (e.g. 1M Tris-HCl, pH 8.5).

RELATED PRODUCTS

Download our Protein Purification and Antibody Production Handbooks.





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Last saved: 2/4/2015 CMH

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