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A Geno Technology, Inc. (USA) brand name

# Amine Magnetic Beads

## (Cat. # 786-906, 786-907)

## INTRODUCTION

G-Biosciences' Amine Magnetic Beads are magnetic beads with an amine (-$NH_2$) surface functional group. The magnetic beads consist of a single-crystal $Fe_3O_4$ sphere core and dextran coating layer. Through chemical modification of dextran, the primary amino group (-$NH_2$) 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.

The magnetic beads with surface-reactive amino groups allow immobilization of ligands such as proteins, peptides, carbohydrates or other target specific molecules. Immobilization of ligands can be through reductive amination of aldehyde or ketones without prior activation of the bead surface. Alternatively, carbodiimide cross-linkers can be used to couple ligands to the amines via their carboxyl groups. Finally, amine reactive heterobifunctional cross-linkers can be used to introduce other functional groups for coupling ligands.

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 |
|---|---|---|
| 786-906 | Amine Magnetic Beads | 1ml |
| 786-907 | Amine 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. Amine group, about 50mM, is coupled covalently to dextran. Amine Magnetic Beads are supplied in phosphate buffered saline, pH 7.4 with 0.09% Sodium Azide and 0.02% Tween-20.

## PRECAUTIONS

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

## PROTOCOL FOR COUPLING THROUGH ALDEHYES & KETONES

Ligands containing aldehydes and ketones can be coupled to amines through Schiff's base formation and reductive amination with sodium cyanoborohydride. Reactive aldehyde groups can be readily prepared by oxidation of glycoproteins with sodium periodate.

***Additional Items Required***

- Coupling Buffer (0.1M sodium phosphate, 0.15M NaCl or 100mM sodium borate, pH9.5 or 100mM sodium citrate, pH9.5)
- 5M Sodium Cyanoborohydride in 1M NaOH (Cat. # 786-061)
- 0.1M Ethanolamine, pH7.4
- Magnetic Stand or magnet

***Preparation of Amine Magnetic Beads***

1. Resuspend the Amine Magnetic Beads thoroughly by pipetting or vortexing the vial for 15 seconds.
2. Transfer adequate amount of Amine 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.

***Binding of Ligand***

1. Dissolve the ligand in the coupling buffer to a concentration of 1-10mg/ml.
2. Add an appropriate volume of ligand to the washed Amine Magnetic Beads and pipette to mix.
3. Add 1µl 5M Sodium Cyanoborohydride in 1M NaOH for every 100µl of reaction mixture. Incubate for 2 hours at room temperature with rocking or rotation.
4. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall.
5. Discard (or collect, if desired) the supernatant as unbound substances, and then remove the tube from the magnetic stand.
6. Add the same volume as reaction mixture of 0.1M ethanolamine to the beads and incubate for 15 minutes at room temperature with rotation.
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. Add an appropriate volume of PBS and resuspend the beads by pipetting.

10. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall.
11. Discard the supernatant, and then remove the tube from the magnetic stand
12. Repeat steps 9-11 twice.

## PROTOCOL FOR COUPLING WITH EDC

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.

***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

***Preparation of Amine Magnetic Beads***

1. Resuspend the Amine Magnetic Beads thoroughly by pipetting or vortexing the vial for 15 seconds.
2. Transfer adequate amount of Amine 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

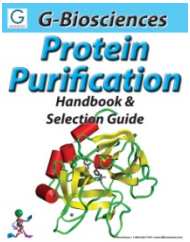
1. Add at least 100µl cell lysate sample containing target antigen to the tube from step 17 and vortex for 10 seconds.
2. Incubate for 30 minutes at room temperature or 4°C overnight with gentle rocking or shaking.
3. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube 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.

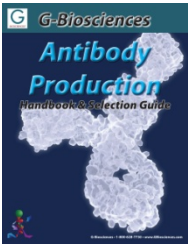
## ELUTION OF ANTIBODY & ANTIGEN

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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