

BcMag™ DADPA-Activated Magnetic Beads

Introduction

BcMag™ DADPA-activated Magnetic Beads are uniform, silica-based superparamagnetic beads coated with high density of DADPA (Diaminodipropylamine) functional groups on the surface. Steroids, dyes, drugs and etc are classes of small molecules, which are difficult or impossible to be immobilized by current immobilization methods due to either absence or inaccessibility of easily reactive functional groups. However, those small molecules may be possibly immobilized to DADPA-terminated magnetic beads via their active hydrogens being condensed with formaldehyde and an amine in the Mannich reaction.

Features and Advantages:

- High binding capacity
- Fast, efficient coupling
- Hydrophilic long-arm spacer minimizing steric hindrance and non-specific binding.



Product Specificities		
Composition	Silica-coated iron oxide magnetic beads grafted with DADPA group on the surface	
Bead Size	~1µm diameter; ~5µm diameter	
Number of Beads	~1.7 x 10 ⁸ beads (1µm beads) /mg; ~5 x 10 ⁷ beads (5µm beads) /mg	
Surface Area	~100 m ² /g	
Stability	Short Term (<1 hour): pH 3-11; Long-Term: pH 4-10 Temperature: 4°C -140°C; Most organic solvents	
Magnetization	~40-45 EMU/g	
Type of Magnetization	Superparamagnetic	
Effective Density	2.5 g/ml	
Formulation	Lyophilized Powder	
Functional Group Density	1µm Magnetic Beads	~200 µmole (1µm beads) / g of Beads
	5µm Magnetic Beads	~180 µmole (5µm beads) / g of Beads
Storage	Store at 4°C upon receipt	

Protocol

Note:

- The following protocol is an example for coupling amine-containing ligands to BcMag™ DADPA-terminated magnetic beads. It is strongly recommended that a titration be performed to optimize the quantity of beads used for each individual application. This protocol can be scaled up and down accordingly.
- The coupling buffer should not contain any amino (e.g. Tris) or formyl groups.
- Coupling efficiency is very low for Ligand containing due to solution phase polymerization

Materials Required

- **Coupling Buffer:** 0.1 M MES, 0.15 M NaCl, pH 4.7
- **Wash Buffer:** 0.1 M Tris, pH 8.0
- **Coupling reagent:** 37% formaldehyde
- **Magnetic Separator (for manual operation):** Based on sample volume, user can choose one of the following magnetic Separators: BcMag separator-2 for holding two individual 1.5 ml centrifuge tubes (Cat. # MS-01); BcMag separator-6 for holding six individual 1.5 ml centrifuge tubes (Cat. # MS-02); BcMag separator-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Cat. # MS-03); BcMag separator-50 for holding one 50 ml centrifuge tube, one 15 ml centrifuge tube, and four individual 1.5 ml centrifuge tubes (Cat. # MS-04) ; BcMag™ separator-96 for holding a 96 ELISA plate (Cat.# MS-05).

A. Sample Preparation

1. Dissolve 1-10mg ligand in 1ml **coupling buffer** if soluble. If insoluble, dissolve it in 0.5 ml 100% Ethanol, then add 0.5 ml coupling buffer to make 50% Ethanol/buffer. (**Note: If Ethanol is used for coupling, the magnetic beads have to be washed with 50% Ethanol prior to adding sample.**)

B. Magnetic Beads Preparation



Note: Weigh and suspend the magnetic beads with 1mM EDTA , pH 7.0 (Concentration: 30mg/ml) and store at 4°C..(Due to small size for 1µm beads, it may be necessary to very gently sonicate the beads to completely disperse the bead.)

1. Shake the bottle to completely resuspend the beads and transfer 30 mg Magnetic beads to a centrifuge tube.
2. Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator. Remove the tube from the separator and resuspend the beads with 1 ml coupling buffer by vortex for 30 seconds.
3. Repeat step-2 two times.

C. Coupling

1. Add sample from A1 and 100 µl coupling reagent to the washed magnetic beads, mix well and incubate at 38-60 °C for >48 hours with continuous rotation.

Note: *User should empirically determine the optimal coupling reaction times and temperatures.*

2. Wash the magnetic beads with 1ml **coupling buffer** for four times and then with d₂H₂O two times as described in B2.

Note:

If insoluble ligand is conjugated in 50% Ethanol, the beads should be first washed with 50% Ethanol three times, then with d₂H₂O two times, then with 100% Ethanol two times, finally with d₂H₂O two times

3. Resuspend the beads in desired buffer containing 0.05% sodium azide and store at 4°C.

D. General Affinity Purification Protocol

1. Transfer optimal amount of the beads to a centrifuge tube. Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator.

Note:

- *It is strongly recommended that a titration be performed to optimize the quantity of beads used for each individual application based on the amount of the target protein in crude sample. Too many magnetic beads used will cause higher backgrounds, while too little beads used will cause lower yields. Each mg of conjugated magnetic beads normally bind to 1-20 µg target protein.*

2. Remove the tube and resuspend the beads with 5 bed bead volume of PBS buffer by vortex for 30 seconds. Leave the tube at room temperature for 1-3 minutes. Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator.
3. Repeat step 2 two times
4. Add washed beads to crude sample containing target protein and incubate at room temperature or desired temperature for 1-2 hours (Lower temperature require longer incubation time).
5. Extensively wash the beads with 5 bed bead volumes of PBS buffer or 1M NaCl until the absorbance of elute at 280 nm approaches background level (OD 280 < 0.05).
6. Elute the target protein by appropriated methods such as low pH (2-4), high pH (10-12), high salt, high temperature , affinity elution or boiling in SDS-PAGE loading buffer.