Steroid/Drug/Dye Conjugation Protocol

Immobilization using traditional procedures may be difficult for molecules lacking easily reactive functional groups. Certain medications, steroids, dyes, and other small chemical molecules, in particular, commonly have structures that lack appropriate reaction groups for immobilization. Other compounds have low-reactivity functional groups or are sterically inhibited. However, some of these compounds contain active (or replaceable) hydrogens that can be condensed using the Mannich method with formaldehyde and amine.

Mannich reaction-induced immobilization

The Mannich reaction is defined as the condensation of formaldehyde (or another aldehyde) with ammonia and another molecule containing active hydrogen. Instead of ammonia, this reaction can be carried out with primary, secondary, or even amide amines. When diaminodipropylamine (DADPA) resin is used as the primary amine in this process, ligand immobilization occurs.

Steroid/Drug/dye Conjugation protocol

The BcMag™ Steroid/Drug/Dye Conjugation Protocol uses DADPA-Terminated Magnetic Beads to efficiently conjugate compounds that lack easily reactive functional groups, such as steroids, dyes, and medications, via Mannich reaction-induced immobilization. BcMag™ DADPA-Terminated Magnetic Beads are uniform, silica-based superparamagnetic beads coated with a high density of DADPA (Diaminodipropylamine) functional groups on the surface. Steroids, dyes, medicines, and other tiny compounds are difficult or impossible to immobilize using present methods due to the absence or inaccessibility of easily reactive functional groups. However, those small molecules may be immobilized to DADPA-terminated magnetic beads via their active hydrogens condensing with formaldehyde and amine in the Mannich reaction.

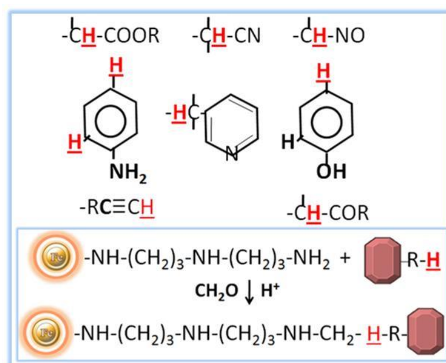

Fig.1 Structure and Coupling reaction of DADPA-terminated magnetic beads

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; ~2.5µm diameter; ~5µm diameter
Number of Beads	• ~ 1.68 x 10^9 beads/mg (1µm beads) • ~ 5x 10^7 beads /mg (5µm beads)
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

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Magnetization	~40-45 EMU/g	
Type of Magnetization	Superparamagnetic	
Effective Density	2.5 g/ml	
Formulation	20 mg/ml (1 mM ETDA, pH 7.0)	
Functional Group Density	1µm Magnetic Beads	~200 µmole / g of Beads
	5µm Magnetic Beads	~180 µmole / g of Beads
Storage	Store at 4°C, protected from light and free of moisture upon receipt	

Protocol

Note:

- *The following protocol is an example for coupling amine-containing ligands to BcMag™ DADPA-terminated magnetic beads. This protocol can be scaled up and down accordingly. Titration is strongly advised to optimize the number of beads needed for each application.*
- *The coupling buffer or ligand 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 rack (for manual operation):** Based on sample volume, the user can choose one of the following magnetic Racks: BcMag rack-2 for holding two individual 1.5 ml centrifuge tubes (Cat. # MS-01); BcMag rack-6 for holding six individual 1.5 ml centrifuge tubes (Cat. # MS-02); BcMag rack-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Cat. # MS-03); BcMag rack-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™ rack-96 for holding a 96 ELISA plate or PCR 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 must be washed with 50% Ethanol before adding the sample*.**)

B. Magnetic Beads Preparation

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 rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack. Remove the tube from the rack 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: ***The user should empirically determine the optimal coupling reaction times and temperatures.***
2. Wash the magnetic beads with 1ml coupling buffer four times and then with dH_2O two times as described in B2.

   Note:

   If the insoluble ligand is conjugated in 50% Ethanol, the beads should be washed with 50% Ethanol three times, dH_2O two times, 100% Ethanol two times, and finally with dH_2O two times.
3. Resuspend the beads in desired buffer containing 0.05% sodium azide and store them at 4°C.

D. General Affinity Purification Protocol

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

   Note:

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

2. Remove the tube and resuspend the beads with a 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 rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack.
3. Repeat step 2 two times
4. Add washed beads to a crude sample containing the 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 eluting at 280 nm approaches the background level ($OD_{280} < 0.05$).
6. Elute the target protein by appropriate methods such as low pH (2-4), high pH (10-12), high salt, high temperature, affinity elution,or boiling in SDS-PAGE loading buffer.

Related Products	
Amine-Terminated Magnetic Beads	Iodoacetyl-Activated Magnetic Beads
DADPA-Activated Magnetic Beads	Peptide conjugation buffer Kit-I
Carboxyl-Terminated Magnetic Beads	Peptide conjugation buffer Kit-II
Epoxy-Activated Magnetic Beads	DVS-Activated Magnetic Beads
Hydrazide-Terminated Magnetic Beads	NHS-Activated Magnetic Beads
Glycoprotein and Antibody Conjugation Kit-I	Hydroxyl-Terminated Magnetic Beads
Glycoprotein and Antibody Conjugation Kit-II	Sulfhydryl-Terminated Magnetic Beads
Aldehyde-Activated Magnetic Beads	Tosyl-Activated Magnetic Beads
Silica-Modified Magnetic Beads	CDI-Activated Magnetic Beads
Alkyne-Activated Magnetic Beads	Thiol-Activated Magnetic Beads
Azide-Activated Magnetic Beads	Cleavable NHS-Activated Magnetic Beads
Cleavable Amine-Terminated Magnetic Beads	Cleavable Azide-Activated Magnetic Beads
Cleavable Carboxyl-Terminated Magnetic Beads	Cleavable Alkyne-Activated Magnetic Beads
Cleavable Epoxy-Activated Magnetic Beads	Cleavable Iodoacetyl-Activated Magnetic Beads
Cleavable Hydrazide-Terminated Magnetic Beads	Cleavable Tosyl-Activated-Magnetic Beads
Cleavable Aldehyde-Activated Magnetic Beads	Streptavidin Magnetic Beads
Boronate Affinity Magnetic Beads	Cleavable Streptavidin Magnetic Beads
Monomer Avidin Magnetic Beads	