DVS-Activated Magnetic Beads

BcMag™ DVS-Activated Magnetic Beads are pre-activated and uniform beads coated with high-density DVS (Divinyl Sulfone) functional groups on the surface. The beads can covalently conjugate primary amino, sulfhydryl, or hydroxyl groups-containing ligands (Fig.1). The matrix is unique affinity support for immobilizing sugars and carbohydrates through the hydroxyl groups present on carbohydrates. The hydrophilic surface ensures low nonspecific adsorption, excellent dispersion, and easy handling in various buffers. BcMag™ DVS-activated magnetic beads are an ideal affinity matrix for immobilizing large molecules or small peptides because the long-arm hydrophilic linker may reduce steric hindrance.

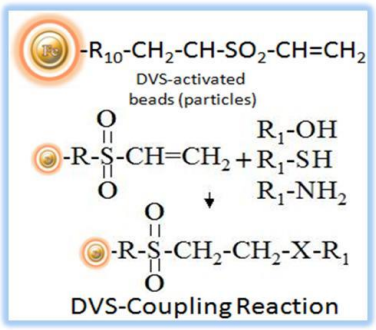

Fig.1 Coupling reaction of DVS-activated magnetic Beads.

Workflow

BcMag™ DVS-activated magnetic beads work perfectly as affinity resin for a wide variety of affinity purification to refine molecules, cells, and parts of cells into purified fractions. After conjugation with ligands, add the beads to a sample containing the target molecules, then mix, incubate, wash and elute the target molecules (Fig.2)

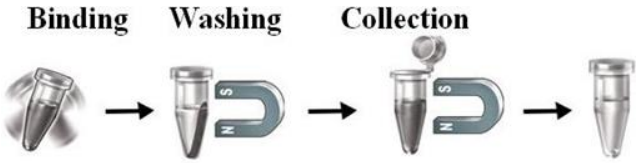

Fig.2

Features and Advantages:

- Pre-activated and ready-to-use
- Easy to use.
- Stable covalent bond with minimal ligand leakage
- Produces reusable immunoaffinity matrix.
- Low nonspecific binding
- Immobilize 15-20 µg protein /mg beads.
- Applications: Immunoprecipitation, Purification for Antibodies, Proteins/Peptides, DNA/RNA

Specification	
Composition	Magnetic Bead grafted with DVS group on the surface
Number of Beads	~ 1.68 x 10^9 beads/mg (1µm beads) ~ 5 x 10^7 beads /mg (5µm beads)
Stability	Short Term (<1 hour): pH 3-11; Long-Term: pH 4-10

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	Temperature: 4°C -140°C; Most organic solvents	
Magnetization	~40-45 EMU/g	
Type of Magnetization	Superparamagnetic	
Formulation	Lyophilized Powder	
Functional Group Density	1µm Magnetic Beads	~250 µmole / g of beads
	5 µm Magnetic Beads	~200 µmole / g of beads
Storage	Ship at room temperature. Store at 4°C upon receipt.	

Protocol

Note:

- *The following protocol is an example for coupling protein and peptides to BcMag™ DVS -Activated magnetic beads. We strongly recommend performing a titration to optimize the concentration of beads used for each application. This protocol can be scaled up and down accordingly.*
- *Coupling buffer containing an appropriate amount of PEG (PEG 20,000) may enhance coupling efficiency and coupling capacity, such as 5 - 7% PEG (w/v, final concentration) for antibody conjugations and 7-10% PEG (w/v, final concentration) for other proteins conjugation.*

Materials Required

- Coupling Buffer : 0.5 M $NaHCO_3$
- Wash Buffer: 0.1 M Na_2HPO_4, 0.5 NaCl, pH 7.2
- Block Buffer: 1 M ethanolamine, pH 9.0
- 30% PEG 20,000 solution
- 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. Land preparation

Note:

- *Coupling efficiencies to DVS-activated magnetic beads vary from ligand to ligand. The user should empirically optimize the concentration of the ligand. Recommend 0.5-10 mg/ml for protein or 200 µmoles ligands per ml for small peptide.*
- *Avoid tris or other buffers containing primary amines or other nucleophiles because these will compete with the intended coupling reaction. But the wash or storage buffers can contain amino.*

1. Dissolve 0.5-10mg protein/peptide in 1ml coupling buffer containing appropriate PEG. If samples have already been suspended in other buffers, dilute samples with an equal volume of coupling buffer.

B. Magnetic beads preparation

1. Prepare 3% magnetic beads with 100% isopropanol (30 mg/ml).
   Note. *It has been stabling for over a year. Store the unused beads in isopropanol solution at 4℃.*
2. Transfer 100 µl (3mg) magnetic beads to a centrifuge tube.
3. 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.
4. Repeat step 3 two times.
5. Remove the supernatant, and the washed beads are ready for coupling.
   Note: *Once rehydrated, use the Bead as soon as possible due to the stability of the functional group.*

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C. Coupling

1. Add 100 µl of ligand solution to the washed magnetic beads and incubate overnight at room temperature with continuous rotation.
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 wash buffer by vortex for 30 seconds. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack.
3. Block the excess active groups on the beads by suspending the beads in 1ml Block buffer and incubate 30-60 minutes at room temperature with continuous rotation.
4. Wash the beads with 1ml Wash buffer four times as described in C2.
5. Resuspend the beads in PBS buffer containing 0.05% sodium azide and store them at 4°C.

D. General affinity purification Protocol

Note:

- *This protocol is a general affinity purification procedure. Designing a universal protocol for all protein purification is impossible because no two proteins are precisely alike. The user should determine the optimal working conditions for purifying the individual target protein to obtain the best results.*
- *We strongly recommended titration 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 magnetic beads typically binds to 10-20 µg of the target protein.*

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.
2. Remove the tube and wash the beads with 5-bed volumes 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 the crude sample containing the target protein and incubate at room or desired temperature for 1-2 hours (Lower temperatures require longer incubation time).

   Note: *Strongly recommended to perform a titration to optimize incubation time. More prolonged incubation may cause higher background.*
5. Extensively wash the beads with 5-beads volumes of PBS buffer or 1M NaCl until the absorbance of eluting at 280 nm approaches the background level ($OD_{280} < 0.05$).

   Note: *Adding a higher concentration of salts, nonionic detergent, and reducing agents may reduce the nonspecific background. For example, adding NaCl (up to 1-1.5 M), 0.1-0.5% nonionic detergents such as Triton X 100 or Tween 20, and a reducing reagent such as DTT or TCEP (we usually use 3mM) to the washing buffer.*
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 an SDS-PAGE sample buffer.

   Note: The linkage chemistry of DVS coupled ligand is unstable in alkaline conditions; acid elution is recommended when the pH elution method is desired.

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	