Boronate Affinity Magnetic Beads

Introduction

Affinity purifications are popular tools for the separation of biological compounds. Among them, boronate affinity chromatography is unique because the ligand (m-aminophenyl boronic acid) specifically and reversibly binds the vicinal diol of *cis*-diol-containing compounds at pH 8-9 and dissociates at pH 3-4 or addition of sorbitol. The boronate affinity resin is a powerful tool for efficiently enriching cis-diol-containing compounds such as alcohols, nucleosides, nucleotides, nucleic acids, carbohydrates, glycoproteins, and enzymes from complex samples such as human serum. Although current boronate affinity-based different chromatography matrices such as columns are available, their procedures are tedious, time-consuming, and unable to handle very tiny samples such as cancer-cell targeting and single-cell analysis. We developed a novel, efficient magnetic affinity system to overcome these limitations.

BcMag™ Boronate Affinity Magnetic Beads are inert silica-enclosed magnetic beads grafted with a high density of borinic acid groups on the surface. The beads work perfectly as solid resin for various affinity purifications of cis-diol-containing compounds. Due to its magnetic property, the magnetic matrix can easily fit into an automation system for high throughput assays.

Workflow

The affinity purification protocol is straightforward (Fig.1). 1. Add the beads directly to the sample. 2. Pipette or vortex to capture the target biomolecules. 3. Magnetic separation of the beads from the sample. 4. Wash to remove the nonspecific binding molecules. 5. Elute the target molecules. The easy-to-use magnetic beads significantly improve results over the standard drip column and batch methodologies with minimum samples.

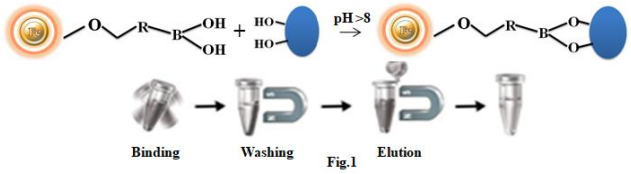

Fig.1

Features and Advantages

- Simple protocol and Easy to use
- Low nonspecific Binding
- High binding capacity and elute bound vicinal diol group containing molecules in mile conditions.
- Easy to use
- Reliable and reproducible results
- Cost-effective: Eliminates columns, filters, and laborious repeat pipetting
- High throughput: Compatible with many different automated liquid handling systems

Specification	
Composition	Silica-enclosed magnetic beads grafted with m-aminophenyl boronic acid
Bead Size	5μm diameter
Magnetization	~40 EMU/g
Type of Magnetization	Superparamagnetic
Effective Density	2.5 g/ml
Formulation	Lyophilized Powder
Binding Capacity	~ 2μmol boronate/mg
Storage	Upon receipt, store at 4°C

PROTOCOL

Materials Required by the User

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- Binding/Wash Buffer: 50 mM HEPES, pH 8.5

  Note: *It may increase the binding capacity for some glycoproteins by adding 20–50 mM Mg^{2+} to the binding buffer. However, it may cause higher nonspecific Binding. We recommended performing a titration to optimize the concentration of Mg^{2+}.*

- Elution Buffer:100 mM sorbitol, 50 mM HEPES, pH 8.5
- Magnetic rack

Item	Source
Magnetic rack for centrifuge tube ** 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 (Bioclone, Cat. # MS-01) • BcMag rack-6 for holding six individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-02) • BcMag rack-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Bioclone, 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 (Bioclone, Cat.# MS-04)
BcMag 96-well Plate Magnetic Rack.	• BcMag 96-well Plate Magnetic Rack (side-pull) compatible with 96-well PCR plate and 96-well microplate or other compatible racks (Blioclone, Cat#: MS-06)
Adjustable Single and Multichannel pipettes	

Procedure

IMPORTANT!

- *The following protocol is an example of the purification of glycoprotein. We recommended performing a titration to optimize the amount of Beads used for each application. This protocol can be scaled up and down accordingly*.
- *Do not use buffers containing organic solvents.*

A. Magnetic Beads preparation

1. Resuspend the BcMag Magnetic Beads with Binding/Washing buffer to make 50mg/ml.

   Note: *It is essential to mix the beads before dispensing. Do not allow the beads to sit for more than 2 minutes before dispensing. Resuspend the magnetic beads every 2 minutes.*

2. Transfer desired amount of magnetic Beads to a tube. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack.
3. Remove the tube and resuspend the Beads thoroughly with 10x Binding/Wash Buffer volumes. Leave the tube at room temperature for 2-3 minutes. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack.
4. Repeat step 3 once.
5. Remove the tube from the rack and resuspend the Beads thoroughly with 10x Binding/Wash Buffer volumes. Place the tube on a magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack.
6. Repeat step 5 once.
7. Resuspend the Beads thoroughly with 1x volumes of Binding/Wash Buffer

B. Sample Binding/Washing

1. Dilute sample with 15 x volumes of Binding/Wash Buffer.
2. Add washed magnetic Beads to the diluted sample, mix Beads well with a pipette and leave them at room temperature for 10 minutes with gentle shaking.
3. Place the tube on a magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack.

   Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack. 4. Remove the tube from the rack and wash the Beads with 15 x Binding/Wash Buffer volumes.
5. Repeat step 3 until the absorbance at 280 nm reaches 0.001.
6. Resuspend the Beads thoroughly with 1x volumes of Binding/Wash Buffer

C. Elution

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1. Remove the tube from the rack.
2. Resuspend the Beads with 10-20 µl Elution Buffer and elute the target biomolecules by pipetting up and down 20-30 times.
3. Place the tube on the magnetic rack for 1-3 minutes and transfer the supernatant containing the eluted protein to a new tube.

D. Trouble Shooting

Problems	Possible Causes	Suggestion
Low yield	The pH in the binding buffer or sample is not within 8.0–8.5.	Check the binding and sample pH to make sure it falls within 8.0–8.5
Non-Binding	Some specific glycosylated proteins are poorly binding to the Beads.	Add additional optimal concentration of Mg^{2+} to binding buffer
Nonspecific Binding	• Excess amount of Beads added to the sample • Washing is not enough. • The sample contains sugar or non-target vicinal diol group containing molecules.	• Optimization of the ratio of sample to particle • Washing the sample until the absorbance at 280 nm reaches 0.001. • Increase NaCl concentration in Bing/Washing buffer. • Run a PD-10 column to remove low molecular weight sugars.

Related Products	
Glycoprotein and Antibody Conjugation Kit-I	Peptide conjugation buffer Kit-I
Glycoprotein and Antibody Conjugation Kit-II	Peptide conjugation buffer Kit-II
Protein A and G Purification Kit	Quick Albumin Removal Kit
Protein A Magnetic Beads Purification Kit	Quick HSA and IgG Depletion Kit
Protein G Magnetic Beads Purification Kit	Quick Antibody Purification Kit
Protein L Purification Kit	One-Step Antibody Purification Kit
Protein A and G Europium Fluorescent Magnetic Beads	Protein G Europium Fluorescent Magnetic Beads
Protein A and G Terbium Fluorescent Magnetic Beads	Protein G Terbium Fluorescent Magnetic Beads
Protein A and G Ruthenium Fluorescent Magnetic Beads	Protein G Ruthenium Fluorescent Magnetic Beads
Protein A Europium Fluorescent Magnetic Beads	Protein L Europium Fluorescent Magnetic Beads
Protein A Terbium Fluorescent Magnetic Beads	Protein L Terbium Fluorescent Magnetic Beads
Protein A Ruthenium Fluorescent Magnetic Beads	Protein L-Ruthenium Fluorescent Magnetic Beads

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