Monomer Avidin Magnetic Beads

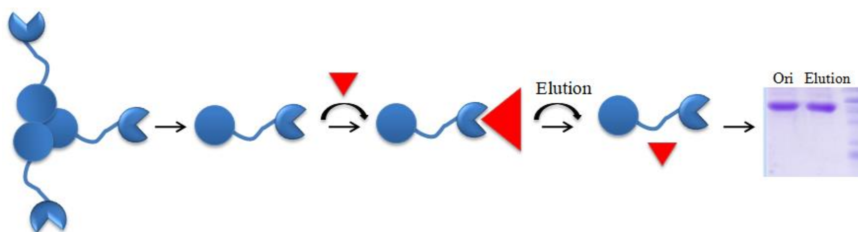
Introduction

BcMag™ Monomer Avidin Magnetic Beads are highly uniform superparamagnetic microspheres coated with high purity density (>97%) avidin subunit monomer on the surface. Monomeric avidin, derived from the native tetrameric protein, retains the same biotin-binding specificity as native avidin, but its biotin-binding affinity dramatically decreases (kD= ~10^{-8} M). Therefore, the bound molecules can be easily eluted from the beads by mild elution conditions such as 2mM biotin instead of harsh elution reagents such as 8M guanidine or SDS detergent. The beads perfectly fit into applications such as immunoprecipitation, cell sorting, and rapid single-step capture of biotinylated molecules such as DNA, RNA, antibody, or protein from cell lysates or hybridization reactions.

The interaction between avidin (or streptavidin) and biotin exhibits one of the highest known non-covalent interactions). Avidin, streptavidin, monomeric avidin, and their analogs have become powerful tools for probes and affinity ligands for various applications in biochemical assays, diagnosis, affinity purification, and drug delivery.

Features and Benefits

1. Quick, Easy, and one-step high-throughput procedures; eliminates columns or filters or laborious repeat pipetting or centrifugation (Fig.1)
2. High binding capacity and elute bound biotinylated molecules in mild conditions.
3. Purifies biotinylated products under mild elution conditions
4. Exhibits little nonspecific binding
5. Scalable - easily adjusts for sample size and automation
6. Low cost: Up to half the price of competitive magnetic beads
7. Beads can be reused at least five times



Specification		
Bead Size	2.5µm diameter	
Number of Beads	~1.47 x 10^8 beads/mg	
Magnetization	~40-45 EMU/g	
Type of Magnetization	Superparamagnetic	
Concentration	10 mg/ml (10mM Tris, 0.15 M NaCl,0.1% BSA,1 mM EDTA, pH7.4)	
Binding Capacity	Biotinylated BSA / ml of Beads	>1mg/ml
	Biotinylate single-stranded oligonucleotides	~ 2,000 pmoles /ml
Storage	Ship at room temperature. Store at 4°C. Do not freeze	

Protocol

Materials Required

Buffer

- 1x PBS Buffer (0.1 M sodium phosphate, 0.15 M sodium chloride; pH 7)
- 1x Regeneration Buffer (0.1 M Glycine/HCl, pH 2.8)
- 1x Blocking/Elution Buffer (2 mM D-biotin in PBS)

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 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)

Protocol

The protocol can be adequately scaled up or down.

Note: *Before purifying biotinylated proteins, peptides, and other molecules. The user should equilibrate all the reagents contained in the kit to room temperature and make 1x working solutions with double-distilled H_2O.*

1. Gently shake the Magnetic Beads bottle until the magnetic beads are entirely suspended—transfer 50 µl beads to a fresh tube.
   Note: *Each user should empirically determine the optimal amount of beads to be used based on the amount of the biotinylated molecules in the crude sample. Too many magnetic beads will result in a higher background; too little will reduce the yield. We recommend 50 µ l of the wholly suspended beads per 50 µg of biotinylated molecules.*
2. Place the tube on a magnetic rack for 1 minute. Remove the supernatant while the tube remains on the Rack. Remove the tube from the Rack. Add four bead volumes of dH_2O mix well and place the tube on the magnetic Rack for 1 minute. Remove the supernatant while the tube remains on the Rack.
3. Wash the beads with four bead volumes of 1x PBS buffer as described in step 2.
4. Add three bead volumes of 1x Blocking / Elution Buffer, mix well by vortex, and incubate at room temperature for 5 minutes. Place the tube on the magnetic Rack for 1 minute. Remove the supernatant while the tube remains on the Rack.
5. Add six bead volumes of 1x Regeneration Buffer, mix well by vortex, and place the tube on the magnetic Rack for 1 minute. Remove the supernatant while the tube remains on the Rack.
6. Add four bead volumes of 1x PBS Buffer and wash the beads as described in step A.2. The beads are ready to use
   Note: *Use the beads immediately, or binding capacity will dramatically reduce the binding capacity.*
7. Add biotinylated molecules-containing sample, mix well by pipetting and incubate at room temperature for 30-60 minutes with gentle rotation.
8. Place the tube on a magnetic rack and remove the supernatant while the tube remains on the Rack. Wash the beads with 1x PBS as in step 2 until the absorbance of eluting at 280 nm approaches the background level ($OD_{280} < 0.05$).
9. Add one bead volume of Blocking/Elution Buffer, mix well by pipetting several times, and incubate at room temperature for 5-10 minutes to elute the bound biotinylated molecule from the magnetic beads.

Related Products	
Boronate Affinity Magnetic Beads	Streptavidin Magnetic Beads
Monomer Avidin Magnetic Beads	Cleavable Streptavidin Magnetic Beads
Streptavidin Europium Fluorescent Magnetic Beads	Avidin Europium Fluorescent Magnetic Beads
Streptavidin Terbium Fluorescent Magnetic Beads	Avidin Terbium Fluorescent Magnetic Beads
Streptavidin-Ruthenium Fluorescent Magnetic Beads	Avidin Ruthenium Fluorescent Magnetic Beads