Mono-avidin Beads

Cat. 1009-1/5

Reversible support for immobilization/purification of biotinylated ligands

ver. 1.1

Introduction

The **Mono-Avidin Beads** manufactured by Adar biotech enables mild affinity purification of biotinylated proteins, peptides and other ligands. The mono-avidin molecules immobilized on the beads have a much lower biotin-binding affinity than native avidin thus enabling dissociation of biotinylated molecules using free biotin molecules or low pH buffer. In addition, the **Mono-Avidin Beads** can be regenerated at least 8 times with insignificant loss in binding capacity.

Mono-Avidin Beads specifications

Matrix: Sepharose™ CL-4B
Type of Avidin bound to beads: Mono-Avidin
Binding capacity: 2-3 mg biotinilated BSA per ml of beads
Bead size: 40-140 µm
Bead structure: Highly cross-linked spherical agarose, 4%
Max back pressure: 0.3 MPa, 3 bar
Max. flow rates: 4 ml/min/cm^2
Recommended flow rate: 1-2 ml/min/cm^2
Stability of the matrix: pH 2-11.
Storage: 4°C in PBS pH 7.4 added with NaN_3 0.05% (w/v) as a preservative.

Protocol: Immobilization of biotinylated ligand

A. Buffers needed

Equilibration and wash buffer: Phosphate buffer saline (PBS) pH 7.4

Elution buffer (200 ml): 2 mM D-biotin in Phosphate buffer saline

Regeneration buffer (250 ml): 0.1 M glycine, pH 2.8

Storage buffer: Phosphate buffer saline (PBS) pH 7.42 plus 0.05% sodium azide as preservative

B. Preparation of beads to work in column:

1. Mix the **Mono-Avidin Beads** slurry thoroughly until homogeneous suspension is visible. Transfer the required amount of gel suspension into an appropriate column with inner diameter of 1.0 to 1.5 cm.
2. After column preparation equilibrate the column with Equilibration buffer by washing with 5-10 column volumes. Recommended flow rates are 1-2 ml/min/cm^2.

C. Protein purification on column:

1. Prior to applying the biotinylated sample, remove extraneous sources of un-reacted biotin by dialysis or gel filtration.
2. Apply the biotinylated sample to column. Allow the biotinylated sample to incubate for at least 30 minutes. For incubation, cap the bottom and then the top of the column and incubate at room temperature at room temperature.
3. After incubation, remove caps and wash the unbound sample 5-10 column volumes with Wash buffer. Now, the affinity support is ready for use.
4. After using the biotinylated sample for affinity purification wash the beads with 5-10 column of Wash buffer.
5. To elute the bound biotinylated molecule, wash the beads with 3-5 volumes of Elution Buffer followed by 3-5 volumes of Wash buffer. Collect the effluent in different fractions and measure the absorbance of each fraction at 280 nm (use PBS to obtain a baseline value).

D. Regeneration and Storage of Mono-Avidin Beads

1. Regenerate column by washing with 3-5 volumes of Regeneration Buffer.
2. For storage, wash column with 3-5 volumes of Storage buffer. Store beads at 4°C.