



Imino-Biotin Beads

Cat. 1011-1/5

Reversible support for the purification of Avidin

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Introduction

The **Imino-Biotin Beads** manufactured by Adar biotech enables mild affinity purification of various proteins belonging to the Avidins family. The Imino-Biotin molecules immobilized on the beads are capable of binding Avidins at neutral and basic pH and eluting in under acidic conditions.

The **Imino-Biotin Beads** can be regenerated at least 10 times with no significant loss in binding capacity.

Imino-BiotinBeads specifications

Matrix: Sepharose™ CL-4B

Type of coupling of Imino-Biotin to beads: Epoxy

Binding capacity: 2-2.6 mg Avidin 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/cm²

Recommended flow rate: 1-2 ml/min/cm²

Stability of the matrix: pH 2-11.

Storage: 4°C in PBS pH 7.4 added with NaN₃ 0.05% (w/v) as a preservative.

Protocol: Immobilization of biotinylated ligand

A. Buffers needed

Equilibration and wash buffer: 50 m M Na₂CO₃, pH 9.8, 0.5 M NaCl

Elution buffer (200 ml): 0.1 M acetic acid, pH 2.9

Regeneration buffer (250 ml): 0.1N glycine pH 2.6 (adjust pH by 2N HCl)

Storage buffer: Phosphate buffer saline (PBS) pH 7.42 plus 0.1% sodium azide as preservative

B. Preparation of beads to work in column:

1. Mix the **Imino-Biotin 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/cm².

C. Protein purification on column:

1. Apply clarified protein containing sample in 0.1M Na₂CO₃, pH 9.8, 0.5 M NaCl on the column at 0.5-1.0 ml/min.
2. Wash column with 20 bed volumes of the wash buffer.
3. Elute Avidin by applying 10 bed volumes of the acidic elution buffer. Dialyze protein containing fractions immediately against x200 volumes of the equilibration buffer. Apply 3 changes of the dialysis buffer spaced by at least 4 hours for completing the dialysis.

D. Regeneration and Storage of Imino-BiotinBeads

1. Regenerate column by washing with 10 volumes of Regeneration Buffer.
2. For storage, wash column with 3-5 volumes of Storage buffer. Store beads at 4°C.