FT-90968A

Monomeric Avidin-Agarose

Reagent for the purification of biotinylated molecules

Product Information

Name: Avidin immobilized onto an agarose gel

Part Number : 90968A 5ml

Matrix : Sepharose® CL-4B
(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.

Immobilized monomeric avidin: Approx. 3 mg per ml of wet gel

Binding capacity: 30 mg of d-biotin per ml of wet gel

Form: Suspension in PBS pH 7.4 ; NaN3 0.1 %(w/v)

Storage: +4°C. DO NOT FREEZE

Technical information

Uptima offers this monomeric avidin-agarose reagent for R&D applications in vitro use :
-immunopurification of biotinylated molecules
Note : for immobilization of biotinylated peptides,antibodies… notably for the preparation of affinity gels, or for removing biotin and biotinylated molecules from samples, see avidin-agarose product #34090A.

Avidin, a 67 kDa tetrameric protein purified from eggs, exhibits a very strong binding capacity with biotin, a 244 kDa vitamin. The incomparable affinity, the highest known among biomolecules, is favourably put to good account for detection purposes. However the dissociation of avidin from biotinylated molecules requires very harsh conditions (6M Guanidine for example) that are incompatible with separation and purification applications.
Monomeric avidin is the monomeric form of avidin, that has a reduced affinity for biotin, allowing milder elution conditions.

- MonomericAvidin is linked covalently to 4% crosslinked agarose, to ensure high biotin capacity but low unspecific binding and low bleaching of avidin. The coupling of avidin ensures very good stability, and very low bleaching. The gel can thus be used 10 to 20 times without a notable decrease of the binding capacity. When properly stored, the gel is stable at least 1 year.
- The capacity of binding is 30 mg of d-biotin per ml of wet gel.
- The dissociation of avidin from biotinylated molecules requires very harsh conditions (6M Guanidine for example). Under classical conditions, the immobilized molecule remain in place so it can be used for the purification of its ligands : complex samples are incubated with an immobilized probe, then ligands bond onto the column can be eluted under alkalin, acidic, or chaotropic conditions.
- Avidin gel can be used in batch or in packed columns. Batch is convenient for analytical separations ('immunoprecipitation') from different and complex samples, while columns are preferred for repeated uses.
- Applications.

- purification of biotin containing enzymes
- purification of receptors through a biotinylated ligand
- preparation of mono-biotinylated molecules
- removing of biotin or biotinylated molecules from samples

Directions for use

Protocol of 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 7.4, plus 0.1% 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/cm2.

C. Protein purification on column:

1. Prior 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.

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.

Legals

For R&D use only.

Related products

See BioSciences Innovations catalogue and e-search tool.

*Associated products:
Buffers, i.e. PBS #UP68723A
Disposable plastic columns PBS buffer #30715A D-Biotin 10685E Glycine 018225
Biotinylation reagents pages B41+, i.e. NHS-PEO-Biotins UPR2027A

*other reactive supports
Hydrazide-Agarose #AWJK30 reactive support (by hydrazide groups)
AmiRGel-Agarose #56408A: amino reactive support (by aldehyde groups)
DVS Activated agarose #WU6750: amino or hydroxyl reactive support

*Immobilized ligands supports:
Enzymes: pepsin #49978A Heparin #35692
IgG binding proteins: Protein A#UP904670, Protein G#UP29337, ProteinL #U0780,
Biotin binding proteins: Avidin #34090A, Streptavidin #51559A, Monomeric avidin #UP29337 , iminobiotin UP88722A

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