FT-88722A

Biotin- and IminoBiotin-Agarose

Product Information

Name:	Biotin immobilized on agarose gel	Imino-Biotin immobilized on agarose gel
Part Number :	**UP88722A**, 5ml	**UP39071A**, 5ml
Matrix :	Sepharose® CL-4B (Highly cross-linked spherical agarose, 4%; 40-140µm beads) 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.	
Binding capacity:	2-2.6 mg Avidin per ml of beads	
Form:	Suspension in PBS pH 7.4; NaN3 0.05 %(w/v)	
Storage:	+4°C (L)	

Technical Information

Uptima offers biotin-agarose reagents for various R&D applications *in vitro use*:

- purification of avidin and streptavidin from eggs or bacterial samples
- removing of free avidin from (strept)avidin conjugates preparations
- purification of avidin conjugates (imminoBiotin-agarose)
- immobilization of (strept)avidin-ligand conjugate for affinity purifications

d-Biotin, a vitamin of 244 Da, that shows very affine interaction with avidin and streptavidin molecules, the highest known among biomolecules (Ka=10^{-15} M^{-1} L^{-1} with avidin, and 10^{-14} with streptavidin). This is taken to good account for capture and immobilization purposes. Information is available in technical sheet #FT-10685A.
The **IminoBiotin**, a derivative of biotin, shows a pH dependent binding that allows milder elution in the case or iminoBiotin. Information is available in the technical sheet #FT-10685A.
d-Biotin and ImminoBiotin are coupled covalently to an agarose gel to ensure excellent stability and high binding capacity. Avidin (Uptima product # UP39860) is a 67 kDa tetrameric protein purified from eggs, and Streptavidin (product #UP51558) is extracted from *Streptomyces avidinii.* Information is available in technical sheet #FT-51558C.

- Biotin is linked covalently to 4% crosslinked agarose, to ensure high (strept)avidin binding capacity but low unspecific binding and low bleaching of biotin. The coupling ensures very good stability, and very low bleaching. The gel can thus be used 10 to 20 times without a noticable decrease of the binding capacity. When properly stored, the gel is stable at least 1 year.
- The capacity of binding is 10 mg of avidin per ml of wet gel. This should be used to evaluate the quantity of sample to be applied on the gel.
- Incubation of 15 to 30 min are usually sufficient for complete binding. The sample to be purified should be dialysed (use CelluSep membranes) against an alkaline buffer (Orr 1981) prior to gel contact, to ensure efficient binding. One may use 400mM NaCl buffered by 100 mM Tris, ammonium or carbonate at pH 10. Depending on the avidin conjugate stability with pH, binding may be sufficient at pH 9, or strenghtened at pH up 11.

For any question,
contact your local distributor

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- The dissociation of avidin from biotinylated molecules requires very harsh conditions (4 M Urea, or >6 M Guanidine pH 1.5). 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.
- The dissociation of (strept)avidin from iminoBiotin is possible at pH4.0. One could use for example 0.1 M citrate or acetate with 400 mM NaCl pH 4.0, or competition by d-Biotin (product #UP10685). Eluted fractions may be neutralized, then desalted (use CelluSep membranes) for further use.
- Biotin-agarose and IminoBiotin-agarose gels can be used in batch or in packed columns (ask Uptima for empty suitable columns). Batch is convenient for analytical separations ('immunoprecipitation') from different and complex samples, while columns are preferred for repeated uses.
- Washes between binding step and elution step may be preformed with any usual buffers, as classically with PBS (NaCl phosphate pH 7.5, product #UP30715), TBS (Tris 10 mM, NaCl 150 mM, pH 7.5), or for more stringent cleaning with 50mM Hepes, pH 7.5, containing 0.5 M NaCl (Hofman 1980) .
- Elution of (strep)avidin labeled proteins can be performed with 50 mM pH 4.0 sodium acetate containing 0.5 M NaCl (Hofman 1980) .

Related Uptima products

Immobilized Monomeric avidin products #UP29337
D-Biotin #UP10685A and other biotins
IminoBiotin reactive albeling agents (NHS-IminoBiotin #UP35329A and others)
Unlabeled and labeled (Strept)avidines # UP51558.
CelluSep dialysis membranes
Buffers, i.e. PBS #UP68723A

Literature

. Hofmann Klaus et al, 1980;77; Proc. Natl. Acad. Sci. 77(8), 4666-4668 ; Iminobiotin Affinity Columns and Their Application to Retrieval of Streptavidin ; Article
. Orr, G.A. (1981). The use of the 2-iminobiotin-avidin interaction for the selective retrieval of labeled plasma membrane components. *J. Biol. Chem.* **256,** 761-766. Abstract
. Zeheb, R., Chang, V. and Orr, G.A. (1983). An analytical method for the selective retrieval of iminobiotin-derivatized plasma membrane proteins. *Anal. Biochem.* **129,** 156-161.

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