

Biotin and IminoBiotin-Agarose

Product Description

Name:	Biotin immobilized on agarose gel Biotin immobilisée sur un gel d'agarose	Imino-Biotin immobilized on agarose gel Imino-Biotin immobilisée sur agarose
Part Number :	UP88722A, 5ml	UP39071A, 5ml
Matrix :	Sepharose® CL-4B	
Ligand:	Approx. 1 mg per ml of wet gel.	
Binding capacity:	Approx. 10 mg of avidin per ml of wet gel.	
Conditioning:	Suspension in PBS pH 7.4; Na ₃ N 0.1 % (w/v)	
Storage:	+4°C (L)	

Technical Information

Uptima offers this biotin-agarose reagent 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, while **IminoBiotin**, a derivative of biotin, shows a far lower affine binding.

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 the technical sheet [FT-UP51558](#).

The incomparable affinity of biotin to (strept)avidin, the highest known among biomolecules ($K_a=10^{-15} \text{ M}^{-1} \text{ L}^{-1}$ with avidin, and 10^{-14} with streptavidin) is put to good account for capture and immobilization purposes. The high but lower affinity of IminoBiotin allows milder elution in the case or iminoBiotin.

- 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 noticeable 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.
- 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 any question,
contact your local distributor

Uptima,
powered by



213 Avenue J.F. Kennedy - BP 1140
03103 Montluçon Cedex - France
Tél. 04 70 03 88 55 - Fax 04 70 03 82 60

uptima@interchim.com Hotline : 33(0)4 70 03 76 06

FT-UP88722

for the purification of its ligands: complex samples are incubated with an immobilized probe, then ligands bond onto the column can be eluted under alkaline, acidic, or chaotropic conditions.

- The dissociation of (strept)avidin from iminoBiotin is possible at pH 4.0. One could use for example 0.1 M citrate or acetate with 400 mM NaCl pH 4.0, and d-Biotin (product #[UP10685](#)). Eluted fractions may be neutralized (use product #UP99543), 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 performed 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).

Related Uptima products

Immobilized Monomeric avidin products ask info [FT-UP29337](#)

Unlabeled and labeled (Strept)avidines ask info [FT-UP51558](#).

CelluSep dialysis membranes, PBS, [D-Biotin](#)

Literature

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.

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.

Hofmann, K., Wood, S.W., Brinton, C.C., Montibeller, J.A. and Finn, F.M. (1980). Iminobiotin affinity columns and their application to retrieval of streptavidin. *Proc. Natl. Acad. Sci.* **77**(8), 4666-4668.

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03103 Montluçon Cedex - France
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