

---

# Streptavidin-Agarose

---

## Product Description

<b>Name:</b>	Streptavidin immobilized onto an agarose gel	
<b>Part Number:</b>	UP51559A	2ml of gel
	UP51559B	5ml of gel
<b>Matrix:</b>	Sepharose® CL-4B	
<b>Binding capacity:</b>	Approx. 22 µmol of d-biotin per ml of wet gel	
<b>Form:</b>	Suspension in 0.1 M Sodium Phosphate, 0.1 M NaCl pH7.4; Na <sub>3</sub> N 0.01% (w/v)	
<b>Storage:</b>	+4°C – <b>DO NOT FREEZE</b>	

## Technical Information

Uptima offers this streptavidin-agarose reagent for various R&D applications in vitro uses:

- immobilization of biotinylated peptides, antibodies, lectins... notably for the preparation of affinity gels (affinity purification, immunoprecipitation)
- removing of biotin and biotinylated molecules from samples

- Streptavidin, a ca 60 KDa tetrameric . The incomparable affinity ( $K_a=10^{-14} \text{ M}^{-1} \text{ L}^{-1}$ ) is favourably put to good account for capture and immobilization purposes.
- Streptavidin is linked covalently to 4% crosslinked agarose, to ensure high biotin capacity but low unspecific binding and low bleaching of streptavidin. 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 ca 22µmol of biotin per ml of wet gel. This should be used to evaluate the quantity of sample to be applied on the gel. For antibodies or peptides, the ratio of biotin can be approximated from the conditions of biotinylation with NHS- and Maleimide-Biotin derivatives, or by quantitation of biotin (ask Uptima).
- Under classical conditions, the immobilized biotinylated molecule remains in place so it can be used for the purification of its ligands: complex samples are incubated with an immobilized probe, then ligands bound onto the column can be eluted under alkaline, acidic, or chaotropic conditions. Besides these immunoprecipitation techniques, the gel can be used to purify biotinylated molecules, but the dissociation of streptavidin from biotinylated molecules requires very harsh conditions (>6M Guanidine for example).
- The Streptavidin 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.

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

## Directions for Use

### Guidelines for immunoprecipitation

The sample from which you want to purify a ligand, should be prepared to allow the interaction of the antibody with the ligand, then the antibody with the streptavidin-agarose gel. 150-500 mM NaCl, 20 mM phosphate, 0.1% SDS, 0.1-1% Nonidet40, 0.1-1% DOC and protease inhibitors may be used. If incompatible concentrations of detergent(s) are necessary for cell lysis or solubilization steps, these may be removed by gelfiltration

You then should have a biotinylated probe, often a specific antibody (if you need to biotinylate your self a probe, ask Uptima for biotinylation reagents; product #UP85262 is usually recommended)

Incubate the sample with ca 2 nmol (300 µg IgG) of biotinylated antibody, 4 hours at room temperature or overnight at +4°C, under constant agitation.

The following incubations and washes of the gel can be performed in batch

Incubate the antibody/sample mixture with 100µl of streptavidin-agarose for 30 min at room temperature or 1 H at +4°C.

Wash the gel with PBS (150-500mM NaCl, 20mM phosphate, pH7.5): incubate the gel with >1ml PBS for 10 min; allow the gel sediment or centrifuge at 1000 g for 1min, discard the supernatant; repeat 4-6 times

The elution of the ligands from the immobilized, add 1ml of citrate or acetate buffer 0.1 M pH 3.0, and incubate for 1-5 min; Centrifuge and pipette the supernatant. This should be neutralized rapidly (for example with the Uptima product UPQ99543 or in 1 M Tris pH 9.0 ) to prevent the degradation of the purified molecules.

The purified fraction could be desalted by dialysis or other means, and analysed as desired.

## Other Information

### Literature:

- Buckie, J.W. and Cook, G.M.W. (1986). Specific isolation of surface glycoproteins from intact cells by biotinylated concanavalin A and immobilized streptavidin. *Anal. Biochem.* 156, 463-472.
- Gretch, D.R., Suter, M. and Stinski, M.F. (1987). The use of biotinylated monoclonal antibodies and streptavidin -affinity chromatography to isolate herpes virus hydrophobic proteins or glycoproteins. *Anal. Biochem.* 163, 270-277.
- Hultman, T., et al. (1991) *Biotechniques* 10(1), 84-93.
- Hultman, T., et al. (1989) *Nucleic Acids Research* 17(13), 4937-4946.
- Hultman, T., et al. (1990) *Nucleic Acids Research* 18(17), 5107-5112.
- Lisanti, M.P., Le Bivic, A., Sargiacomo, M. and Rodriguez-Boulant, E. (1989). Steady -state distribution and biogenesis of endogenous Madin-Darby canine kidney glycoproteins: evidence for intracellular sorting and polarized cell surface delivery. *J. Cell Biol.* 109, 2117-2127.
- Mattson, G. (1995) *J. NIH Research* 7, 86.
- Mitchell, L.G. and Merrill, C.R. (1989). Affinity generation of single-stranded DNA for dideoxy sequencing following the polymerase chain reaction. *Anal. Biochem.* 178, 239-242.
- Stacy, et al. (1991) *Nucleic Acids Research* 19(14), 4004.
- Updyke, T.V. and Nicolson, G.L. (1984). Immunoaffinity isolation of membrane antigens with biotinylated monoclonal antibodies and immobilized streptavidin matrices. *J. Immunol. Meth.* 73, 83-95.

Rev. B07E

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