

Immobilized Monomeric Avidin

Product Description

Reagents for the purification of biotinylated molecules

| <u>Part number</u> | <u>Designation</u> |
|--------------------|---|
| UP29337A | Immobilized mon.avidin kit Contains 2ml gel, 2 columns, 25ml of buffer A and B (qsp 250 ml) for binding, elution and regeneration, 50ml of wash buffer (qsp 500 ml). |
| UP90968A | Monomeric avidin immobilized onto 4% agarose, 2ml Binding capacity: 10ug d-biotin per ml of gel |
| UP90968B | Monomeric avidin immobilized onto 4% agarose, 5ml |
| <u>Storage :</u> | Keep at +4°C (L) Stability is 1 year from purchase date, according to recommended storage conditions |

General Information

Avidin, a 67 kDa tetrameric protein purified from eggs, exhibits a very strong binding with biotin, a vitamin of 244 kDa. The incomparable affinity, the highest known amongst 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.

Uptima obtained an avidin derivative, a monomere, that shows a weaker binding toward biotin, ca 10^5 - 10^6 times lower than avidin. Affinity is conserved, but dissociation of biotin occurs under mild conditions. This is linked covalently to 4% crosslinked agarose, to ensure high biotin capacity but low unspecific binding and low bleaching of avidin. Following an easy preparation step, biotinylated molecules will bind readily, and can then be recovered with excellent yields.

Mon.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 purifications.

This makes a very useful tool for various applications. Uptima Mon.Avidin is the method of choice for:

- immobilization of biotinylated peptides, antibodies...
- 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 in batch: make a 25% slurry with PBS + NaN_3 0.1%. Calculate the quantity of gel sufficient for having a biotin binding capacity adequate for your further analysis (i.e. 100µl of wet gel)(see technical info.)

Perform the same protocol as given for columns (below) but:

- Incubate 100µl of wet gel with 2ml of buffer in tubes under constant agitation.
- Remove the buffer by aspiration after a 30sec centrifugation at 1000g.
- The regeneration step is not necessary, as the gel is usually disposed off.

Protocol in column (method preferred for time savings and reproducibility)

- Pack 1ml columns (provided). Pipet a 50% slurry of gel in the column, closed at its outlet with a cap or a plastic film. Leave the gel sediment, then open the column to allow the buffer to flow through by gravity.

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then wash well with PBS + NaN_3 0.1%. Put a fritted disc on the top of the gel. Store closed at both ends and right, at +4°C.

- Set the column at room temperature, tighed to an adequate support with collecting tube below.
- Open the top of the column .
- Open the bottom of the column; the storage buffer flows through by gravity.

Note that the gel does not dry thanks to an upper porous disk.

- Regeneration of reversible biotin binding sites
Add sequentially:
3x3ml of PBS
2x3ml of buffer A (wait 1min after each addition)
3x3ml of buffer B (wait 1min after each addition)
- Binding step: add the sample by 0.5ml fractions (wait 1 min after each addition)

Notes:

The sample may contain detergents and various molecules without incidence on avidin binding. A preliminary dialysis may however be required if ionic strength and pH are extreme. A 0.45µm filtration of samples increases the column life and reduces further contamination. If the sample is more than 2 ml, proceed by 2ml fractions, collect the unretained fractions, and allow a second passage

The incubation may be increased, or replaced by a on-line 30 min incubation

- Wash the column: add sequentially 3ml fractions of PBS until collected fractions have an $\text{OD}_{280\text{nm}} < 0.05$ or lower. This may need 5 to 15 fractions depending on the nature of the sample.
- Elute biotinylated bound molecules: add sequentially 10x1ml fractions of buffer B and collect eluted fractions in separated fresh tubes; measure OD or analyse by a suitable technic in order to locate the interesting biotinylated molecules. As eluted fraction have an acid pH and contain free biotin and other salts, it should be necessary to neutralize (for example with Tris 0.1M pH9.0) to avoid denaturation, and then dessalt by an adequate mean.
- Regenerate, equilibrate and store the column , wash with:
3x3ml of buffer B (wait 1min after each addition)
3x3ml of PBS + NaN_3 0.1%
- Store the column at 4°C : close the bottom, place 1ml of buffer on the top porous disc, and close the top cap.

Technical Information

- The capacity of binding is 10µg of biotin per ml of wet gel. This should be used to evaluate the quantity of sample to be applied on the gel, or the quantity of purified biotinylated molecules when binding is done at saturation and elution is complete (estimated 80% recovery) (d). 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). For biotinylated cells, the number of targetted molecule per cells can give some idea of the quantity of gel to use.
The coupling of mon.avidin 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. Purification yield and gel longevity are affected by bubbles (pack well, use disgaze buffers).
- The buffer A contains d-Biotin 3mM in order to compete with bound biotinylated molecules, and conservative agents . For specific applications, elution can eventually be done with 3mM Biotin in other buffers suitable for further steps.
- The buffer B contains glycine adjusted to pH 2.8, proprietary agents and preservatives.
- The wash buffer is Phosphate Buffered Saline

Other Information

For any question, please ask Uptima.

References

- (1) Guchait R.B., Polakis S.E., Dimroth P., Stoll E., Moss J., and Lane M.D., Acetyl co-enzyme A system *Escherichia coli* Purification and propeties of the biotin carboxylase, carboxyltransferase, and carboxyl carrier protein components; *Biol.Chem.*, 1974, 249, 6633-6645
- (2) Tucker J, Grisshammer R , Purification of a rat neurotensin receptor expressed in *Escherichia coli*.; *Biochem J* 1996 Aug 1;317(Pt 3):891-899 [LW](#)
- (3) [PubMed](#) Sun HB, Qian L, Yokota H., Detection of abasic sites on individual DNA molecules using atomic force microscopy; *Anal Chem* 73, 2229-2232 (2001) PN42811.

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