### AFFINITY PURIFICATION



## PROCEDURE FOR USE Biotin Agarose Resin

Biotin agarose binds with high affinity to avidin and streptavidin making this resin appropriate for non-reversible binding applications, such as removal of avidin or streptavidin from samples.

In both cases, the interaction (biotin-avidin or biotin-streptavidin) is very strong and the bond is stable at extreme pH, organic solvents and denaturing agents.

Immobilized biotin will release avidin if exposed to 8M quanidine-HCl at pH 1.5 or if boiled in reducing SDS-PAGE sample buffer.

#### **INSTRUCTIONS**

The following procedure is for the removal of avidin/streptavidin from samples.

The following summarized procedure is adapted for the removal of avidin/streptavidin from samples.

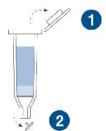
We recommend using our empty columns (Plastic Columns or Plastic Columns XL) depending on total volume size.

#### 1. Elimination of the Preservative

Determine the quantity of Biotin Agarose resin needed for your purification (see technical specifications). Gently shake the bottle of Biotin Agarose resin to achieve a homogeneous suspension. Immediately pipette sufficient suspension to an appropriate empty column <sup>(1)</sup>.

#### (1) Empty column information

Column	Cat. N°	Total capacity
Plastic Columns	C-50	12 ml
Plastic Columns XL	CXL-50	35 ml



Remove first the upper and then the lower cap of the column, to allow elimination of the preservative by gravity flow.

#### 2. Equilibration of the Pre-Packed Column

Equilibrate the column with 5ml bed volumes of binding buffer. Add the binding buffer on the upper part of the column and make sure no air has been trapped. Mix manually inverting the Pre-packed column and discard the supernatant. Repeat the equilibration step twice.

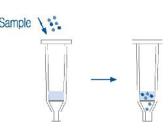
Note: Binding buffer:  $0.1M\ Na_3HPO_4$ ,  $0.15M\ NaCII$ , pH 7.2.



#### 3. Application of the Sample and washing of the Pre-Packed Column

Dissolve or exchange sample into Binding buffer and add it through the top of the column, keeping sample and resin in contact at least 30 minutes before removing the bottom cap. Mix manually inverting the Pre-Packed column. Remove the lower cap of the column and save the entire flow-through.

Note: Binding capacity can be affected by several factors such as sample concentration.



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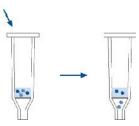
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#### Washing of the Pre-Packed Column

**Binding Buffer** 

Close column outlet with the cap. Add the binding buffer through the top to collect the flow-through.

Note: It will be washed with the binding buffer until the O.D. 280 nm was the same as the binding buffer.



For laboratory use only. Not for use in diagnostic or therapeutic procedures.