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ab270530 Streptavidin Affinity Resin - Amintra

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes ASA0002, ASA0005, ASA0010.

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Streptavidin Affinity Resin - Amintra datasheet:
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For simple, rapid purification of biotin and biotin-containing substances.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Streptavidin Resin - Amintra (ab270530) provides a simple, rapid and reliable method to purify biotin and biotinylated substances.

Purified streptavidin isolated from *Streptomyces avidinii* is immobilized on highly cross-linked 6% agarose. The immobilized streptavidin binds biotin and biotinylated substances and can be used for affinity chromatography applications. The interaction between streptavidin and biotin is very strong and requires denaturing conditions for elution, which may destroy both the ligand and the sample.

Alternatively, it can be used in the purification of antigens, where biotinylated antibodies are incubated with antigen. The biotinylated antibody-antigen complex binds to Streptavidin Resin - Amintra from which the antigen can be eluted. The base matrix of Streptavidin Resin - Amintra is a robust, high cross-linked 6% agarose. The crosslinking of the base matrix has been optimized to give the matrix good flow properties and high physical and chemical stability, both of which are key factors for cost-effective, large-scale use.

2. Materials Supplied and Storage

Store kit at +4°C immediately on receipt. **Do not freeze. Freezing the suspension will damage the agarose beads.** The resin is pre-swollen and defined. It is formulated as a suspension in 20% ethanol.

Item	Quantity			Storage temperature
Streptavidin Resin - Amintra	2 mL	5 mL	10 mL	+4°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Binding buffer (see buffer selection Section 4.1)
- Elution buffer (see buffer selection Section 4.1)

4. Technical Considerations

4.1 Recommended buffers:

Water and chemicals used for buffer preparation should be of high purity. We recommend filtering the buffers by passing them through a 0.22 µm or 0.45 µm filter before use.

Binding of biotin or biotinylated substances:

- Binding buffer: 20 mM NaH_2PO_4, 150 mM NaCl, pH 7.4
- Elution buffer: 8 M guanidine-HCl, pH 1.5

Purification of immunobiotinylated substances

- Binding buffer: 50 mM $(NH_4)_2CO_3$, 0.5 M NaCl, pH 10.0
- Elution buffer: 50 mM NH_4Acetate, 0.5 M NaCl, pH 4.0

4.2 Characterization of the Resin:

Ligand	Streptavidin
Binding capacity	> 120 nmol biotin/mL medium; 6 mg biotinylated serum albumin/mL medium
Particle size	45 µm – 165 µm
Bead structure	Highly cross-linked 6% agarose
Maximum back pressure	0.3 MPa (3 bar, 43 psi)
Recommended linear flow rate*	300 – 500 cm/h
Temperature stability	Short term: 2ºC to room temperature; Long term: 2ºC to 8ºC
Storage buffer	20% ethanol

** Linerar flow rate = volumetric flow rate (cm3/hr)/column cross-sectional area (cm^2)*

5. Assay Procedure

5.1 Sample preparation:

5.1.1 **The sample should be adjusted to the composition of the binding buffer.** This can be done either by diluting the sample with binding buffer or by buffer exchange. The sample should be filtered through a 0.45 µm filter or centrifuged before it is applied to the column.

5.2 Resin preparation:

5.2.1 Streptavidin Resin - Amintra is supplied pre-swollen in 20% ethanol. Wash the required amount of medium with ten volumes of binding buffer to remove the ethanol solution. Prepare a slurry with binding buffer in a ratio of 50% settled medium to 50% buffer.

5.3 Packing Streptavidin Affinity Resin - Amintra:

5.3.1 Remove air from the column dead spaces by flushing the end-piece and adapter with packing buffer. Make sure no air has been trapped under the column net.

5.3.2 Close the column outlet leaving the net covered with packing buffer.

5.3.3 Resuspend the beads stored in its container by shaking (avoid stirring the sedimented medium). Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles.

If using a packing reservoir, immediately fill the remainder of the column and reservoir with packing buffer. Mount the adapter or lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.

5.3.4 Open the bottom outlet of the column and set the pump to run at the desired flow velocity. Ideally, Streptavidin Affinity Resin - Amintra is packed at a constant pressure of approximately 1 bar (0.1 MPa). If the packing equipment does not include a pressure gauge, use a packing flow velocity of approximately 400 cm/hr (10 cm bed height, 25°C, low viscosity buffer). If the recommended pressure or

flow velocity cannot be obtained, use the maximum flow velocity the pump can deliver. This should also give a reasonable well-packed bed. Do not exceed 75% of the packing flow velocity in subsequent chromatographic procedures.

5.3.5 When the bed has stabilized, close the bottom outlet and stop the pump.

If using a packing reservoir, disconnect the reservoir and fit the adapter to the column. If using the column, carefully place the top filter on top of the bed before fitting the adapter.

5.3.6 With the adapter inlet disconnected, push the adapter down, approximately 2 mm into the bed, allowing the packing solution to flush the adapter inlet.

5.3.7 Connect the pump, open the bottom outlet and continue packing. The bed will be further compressed at this point and a space will be formed between the bed surface and the adapter.

5.3.8 Close the bottom outlet. Disconnect the column inlet and lower the adapter approximately 2 mm into the bed. Connect the pump. The column is now ready to use.

5.4 Purification protocol:

1. Fill the syringe or pump tubing with binding buffer. Remove the stopper and connect the column to the syringe (with the provided connector), or pump tubing, "drop to drop" to avoid introducing air into the column. Remove the snap-off end at the column outlet.

7.3.2. Wash the column with 10 column volumes of binding buffer.

7.3.3. Apply the sample, using a syringe fitted to the connector or by pumping it onto the column.

7.3.4. Wash with 5 to 10 column volumes of binding buffer or until no material appears in the effluent.

7.3.5. Elute with 5 column volumes of elution buffer. Other volumes may be required if the interaction is difficult to break.

5.5 Analysis:

Identify the fractions using UV absorbance, SDS-PAGE, or western blot.

*Δ **Note**: The amount of antigen needed, and the incubation time are dependent upon the antibody-antigen system used and may require optimization for each specific system.*

*Δ **Note**: Blocking reagents containing milk products should not be used to block streptavidin agarose due to the presence of endogenous biotin.*

Technical Support

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