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ab270603 Anti-HA Affinity Resin - Amintra

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes: AHA0001, AHA0005

View ab270603 Anti-HA Affinity Resin - Amintra datasheet:

www.abcam.com/ab270603

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For simple, rapid HA-tagged recombinant protein purification and immunoprecipitation.

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

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1. Overview

Anti-HA Affinity Resin - Amintra (ab270603) is designed for simple, rapid HA-tagged recombinant protein purification and immunoprecipitation.

The HA tag is derived from the human influenza virus HA protein corresponding to amino acids 98-106 (YPYDVPDYA). The HA tag does not appear to interfere with the bioactivity or the biodistribution of the recombinant HA-tagged protein, so it has been extensively used as a general epitope tag at the N-terminal or C-terminal of the fusion protein. Anti-HA Affinity Resin - Amintra is based on a 4% agarose beads matrix, and non-specific binding is rare. It can be used for HA fusion protein purification and immunoprecipitation.

2. Materials Supplied and Storage

Store kit at +4°C immediately on receipt. **Do not freeze or store the resin at room temperature.** The resin is pre-swollen and defined. It is formulated in PBS containing 0.02% sodium azide.

Item	Quantity		Storage temperature
Anti-HA Affinity Resin - Amintra	1 mL	5 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 Section 4.2)
- Wash buffer (see Section 4.2)
- Elution buffer (see Section 4.2)
- Neutralization buffer (see Section 4.2)

4. Technical Considerations:

4.1 Characteristics of the Resin:

Matrix	4% agarose
Ligand	Anti-HA Antibody
Binding Capacity	>1mg HA fusion protein/ml medium
Particle Size (µm)	45-165
Maxi Pressure	0.1 MPa, 1 bar
Storage Buffer	1 x PBS with 0.02% NaN_3

- For the purification of HA-tagged fusion proteins, Amintra Anti-HA Affinity Beads can be used in column chromatography, batch format and immunoprecipitation.
- For large volumes of sample, column chromatography or batch format are recommended to quickly capture the target protein from a large volume of extract.
- When a small amount of sample is being purified, immunoprecipitation procedure is recommended.

4.2 Recommended buffers:

Prepare fresh reagents immediately prior to use.

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

- Binding/wash buffer: 10 mM Tris, 0.15 M NaCl, pH 7.4
- Chemical elution buffer: 0.1 M glycine-HCl, pH 2.0-2.8 or 3M NaSCN or 50 mM NaOH
- Competitive elution buffer: 50 mM Tris. 0.15 M NaCl, 100 to 500 µg HA peptide, pH 7.4
- Neutralization buffer: 1 M Tris-HCl, pH 8.5

5. Sample Preparation

To ensure that proper ionic strength and pH are maintained for optimal binding, it is necessary to dilute samples or cell culture supernatant at least 1:1 with Binding/Wash Buffer. Alternatively, the sample can be dialyzed overnight against Binding/Wash Buffer. It is recommended to filter the sample solution by passing them through a 0.22 µm or 0.45 µm filter before use.

Δ Note: *If there are problems with protein stability, it may be desirable to add protease inhibitors to the sample. We recommend Protease Inhibitor Cocktail (ab270050) and Protease Inhibitor Cocktail (EDTA-free) (ab270055).*

6. Assay Procedure

- Equilibrate all materials and prepared reagents to desired purification temperature (4°C or RT) just prior to use and gently agitate.
- For large volumes of sample, column chromatography or batch format are recommended to quickly capture the target protein from a large volume of extract.
- When a small amount of sample is being purified, immunoprecipitation procedure is recommended.

6.1 Column purification:

6.1.1 Mix the slurry by gently inverting the bottle several times to completely suspend the Anti-HA Affinity Beads. Close the column outlet leaving the net covered with packing buffer. Transfer the slurry to the column.

6.1.2 Allow the resin to settle down and the buffer to drain from the column. Add 5 column volumes of Binding Buffer to the column to equilibrate the beads.

6.1.3 Load the sample to the column. Collect the flow-through in order to measure the binding efficiency to the beads, i.e. by SDS-PAGE. We recommend using our RunBlue™ Precast Gels along with InstantBlue Coomassie Stain (ab119211).

6.1.4 Wash the column with 10-20 column volumes of Wash Buffer or until the absorbance of the effluent at 280 nm is stable.

6.1.5 **Chemical Elution:** Elute the target protein with Chemical Elution Buffer and collect the eluate at a fixed volume. Add a tenth of the volume of neutralizing solution to the eluate tube in advance.
Δ Note: *Equilibrate the beads with Binding Buffer after acid elution, do not leave it in the Elution Buffer more than 20 mins.*

6.1.6 **Competitive Elution:** Elute the target protein with Competitive Elution Buffer and collect the eluate at a fixed volume.

6.2 Batch purification:

6.2.1 Resin Preparation: Determine the volume of resin required for your purification. Remove sufficient slurry for use and transfer to an appropriate tube. Drain the storage buffer and equilibrate the beads with 5 column volume of Binding Buffer for cleaning.

6.2.2 Add the sample solution to the prepared Beads and incubate at 4°C or room temperature for at least 1 hour (no magnetic stirring). Use gentle agitation such as end-over-end rotation.

6.2.3 After incubation, sediment the beads by centrifugation at 1000 x *g* for 5 mins or filter the mixture to collect the beads.

6.2.4 Load the Beads into the chromatographic column and wash the column with 10–20 column volumes of Wash Buffer or until the absorbance of the effluent at 280 nm is stable.

6.2.5 Elute with 3–5 column volumes of either **Chemical Elution Buffer** or **Competitive Elution Buffer**.

6.3 Immunoprecipitation:

6.3.1 Completely resuspend Anti-HA Affinity Beads and add 40 µL suspension to 1.5–2 mL micro centrifuge tube. Centrifuge for 30 seconds at 5000 x *g* and discard supernatant.

6.3.2 Add 500 µL of Binding Buffer, centrifuge for 30 secs at 5000 x *g* and discard supernatant. Repeat this step twice.

6.3.3 Add the sample to the tube and gently invert the tube to mix. Incubate the tube at room temperature with mixing (on a shaker or rotator) for at least 1 hour.

6.3.4 Centrifuge for 30 secs at 5000 × *g* and discard supernatant. Repeat this step two times. If necessary, keep the supernatant for analysis.

6.3.5 Add 500 µL of Wash Buffer to the tube and mix well; Centrifuge for 30 secs at 5000 x *g* and discard supernatant. Repeat this step twice.

6.3.6 **Chemical Elution:** Elute the target protein with 100 µL of Chemical Elution Buffer. Incubate the tube at room temperature with mixing (on a shaker or rotator) for 5 mins, centrifuge for 30 secs at 5000 x *g*. Collect the supernatant and add a tenth of the volume of neutralizing solution to the eluate in advance.

6.3.7 **Competitive Elution:** Elute the target protein with 100 µL of Competitive Elution Buffer. Incubate the tube at room temperature with mixing (on a shaker or rotator) for 5 mins, centrifuge for 30 secs at 5000 x *g*. Collect the supernatant.

6.3.8 **Denaturing Elution:** Add 20 µL of 2X SDS-PAGE Sample Buffer to the tube and mix well. Heat the tube at 100°C for 5 mins. Centrifuge for 30 secs at 5000 x *g* and transfer the supernatant containing desired sample into a new tube. Analyze the sample by SDS-PAGE followed by Western Blot Analysis.

6.4 Potential issues:

Issue	Possible cause	Solution
Target protein in flow-through	Beads overloaded	Lower sample volume / increase bead volume
	Binding time too short	Extend incubation time of sample & beads
	Tag not exposed	Add low concentration of denaturant & dialyse before binding
	Reagent incompatibility	Dialyse sample before loading
Target protein not detected in elution fractions	Target protein unstable	Prepare fresh sample. Operate at low temperature. Add protease inhibitors to sample
	No HA fusion protein in sample	Detect HA-tag by Western blot
	Low expression of target protein	Optimise protein expression. Increase sample volume. Reduce NaCl concentration
Background too high	Non-specific binding	Reduce amount of sample. Increase NaCl concentration
	Inadequate washing	Increase washing times and incubate for 5-10 minutes. Increase NaCl concentration in Wash Buffer

Technical Support

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