Anti-HA Affinity Beads

Index

1. Product Description

HA tag (YPYDVPDYA) derived from the 98-106 amino acid of human influenza hemagglutinin protein, which has little influence on the spatial structure of exogenous proteins and is easy to be fused into the N-terminal or C-terminal of target proteins, so it is often used for expression of fusion proteins. **Anti-HA Affinity Beads** consists of 90μm beads of 4% agarose, to which anti-HA antibody has been coupled as affinity ligand. **Anti-HA Affinity Beads** can be used to purify HA tag fusion proteins expressed in prokaryotes, yeast or mammalian cells in one step or for immunoprecipitation.

Table 1. Characteristics of **Anti-HA Affinity Beads 4FF**

Item	Description
Matrix Spherical	4% agarose
Ligand	Anti-HA mouse monoclonal antibody
Static Binding Capacity	>1mg HA fusion protein/ml medium
Particle size	45-165um
Maximum Pressure	0.1MPa, 1bar
Storage Solution	1×PBS containing 0.02% NaN_3
Storage Temperature	2℃-8℃

2. Reagents Prepatration

2.1 Buffer Preparation

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

Binding Buffer: 50mM Tris, 0.15M NaCl, pH7.4

Wash Buffer: 50mM Tris, 0.15M NaCl, 0.05% Tween-20, pH7.4

Elution Buffer: 0.1M Glycine HCl, pH2.0-2.8

Competitive Elution Buffer: 50mM Tris, 0.15M NaCl, 100-500ug HA peptide/ml, pH7.4

Neutralization Buffer: 1M Tris-HCl，pH8.5

2.2 Sample Preparation

To insure 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 0.22μm or 0.45μm filter before use.

3.Purification Procedure

3.1 Column Chromatography

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.

2) Allow the resin to settle down and the buffer to drain from the column. Add 5 column volumes Binding Buffer onto the column to equilibrate the beads.

3) Apply the sample to the column and drain the flow-through. Multiple binding passed may be required for complete binding. Collect the flow-through for measuring the binding efficiency to the beads, i.e. by SDS-PAGE.

4) Wash the column with 10-15 column volumes Wash Buffer or until the absorbance of the effluent at 280 nm is stable.
5) **A Acid Eluton:** Elute the target protein with 5 column volumes Elution Buffer and collect the eluate at a fixed volume. Add a tenth of the volume of neutralizing solution to the eluate in advance.
Notice: Equilibrate the beads with Binding Buffer after acid elution, do not leave it in the Elution Buffer more than 20min.
B Competitive Elution: Elute with 5 column volumes of Competitive Elution Buffer.
6) Regeneration with 3 column volumes of Elution Buffer. Equilibrate the column with 3 column volumes of Binding Buffer , 5 column volumes of distilled water. Finally store the resin with 1XPBS containing 0.02% NaN_3 at 2-8℃.
7) Identify the fractions containing the protein. Use UV absorbance, SDS-PAGE, or western blot.

3.2 Static Adsorption

1) Resin preparation: Determine the volume of resin required for your purification. Remove sufficient slurry for use and transfer to an appropriate tube. Remove the storage buffer and equilibrate the beads with 5 column volume of Binding Buffer thrice.
2) Add the sample solution to the prepared beads and incubate at 4℃ or room temperature for at least 1 hour(no magnetic stirring). Use gentle agitation such as end-over-end rotation.
3) After incubation, sediment the beads by centrifugation at 5000×g for 1 min or filter the mixture to collect the beads.
4) Load the Beads into the column and wash the column with 10-20 column volumes Wash Buffer or until the absorbance of the effluent at 280 nm is stable.
5) Elute the protein refer to the fifth step of 3.1.
6) Regeneration with 3 column volumes of Elution Buffer. Equilibrate the column with 3 column volumes of Binding Buffer , 5 column volumes of distilled water. Finally store the resin with 1XPBS containing 0.02% NaN_3 at 2-8℃.
7) Identify the fractions containing the protein. Use UV absorbance, SDS-PAGE, or western blot.

3.3 Immune Precipitation

1) Completely resuspend **Anti-HA Affinity Beads.** Add 40μl (column volume 20ul) to 1.5-2ml micro centrifuge tube. Centrifuge for 30 seconds at 5000×*g* and discard supernatant.
2) Add 500ul of binding Buffer, centrifuge for 30 seconds at 5000×g and discard supernatant. Repeat this step two times.
3) Add 200-1000ul sample into 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.
4) Centrifuge for 30 seconds at 5000×g and discard supernatant. Repeat this step two times. If necessary, keep the supernatant for analysis.
5) Add 500 μl Wash Buffer to the tube and mix well, Centrifuge for 30 seconds at 5000 × g and discard supernatant. Repeat this step two times.
6) **A Acid Eluton:** Elute the target protein with 100ul Elution Buffer. Incubate the tube at room temperature with mixing (on a shaker or rotator) for 5min, centrifuge for 30 seconds at 5000 × g. Collect the supernatant and add a tenth of the volume of neutralizing solution to the eluate in advance.
B Competitive Elution: Elute the target protein with 100ul Competitive Elution Buffer. Incubate the tube at room temperature with mixing (on a shaker or rotator) for 5min, centrifuge for 30 seconds at 5000 × g and collect supernatant.
C Degeneration Elution: DTT and beta-Mercaptoethanol in the SDS-PAGE sample loading buffer can break the disulfide bond and disconnect the light chain and heavy chain of antibody. Anti-HA antibody can also be denatured by SDS in the SDS-PAGE sample loading buffer. So **Anti-HA Affinity Beads** can not be reused again after denaturing elution.
Add 20μl 2XSDS-PAGE Sample Buffer to the tube and mix well. Heat the tube at 100°C for five minutes. Centrifuge for 30 seconds at 5000 × g and transfer the supernatant containing desired sample into a new tube.
7) Analyze the sample by SDS - PAGE followed by Western blot analysis.

4. Troubleshooting

Problem	Possible Cause	Solution
Target protein in the flow through.	The Beads is over loaded.	Low the sample volume or increase the Beads.
	The binding time is short.	Extend the incubation time of sample and beads.
	Tag is not exposed.	Add low concentration of denaturant and dialysis before loading.
	Reagent incompatibility	Dialysis the sample before loading.

No target protein is detected in any elution fraction.	Target protein is not stable.	Prepare new sample. Operate at low temperature. Add protease inhibitor in the sample solution.
	No HA fusion protein in the sample.	Detect the tag by Western Blot.
	Low expression of target protein.	Optimize the protein expression level. Increase the sample amout. Reduce the concentration of NaCl.
	The sample is not eluted completely.	Use 2-8M urea to elute the sample.
Background noise	Nonspecific adsorption	Reduce the sample amount, increase the concentration of NaCl.
	Inadequate washing	Increase the washing times and incubate for 5-10 minutes. Increase the concentration of NaCl in the Wasing Buffer.

5. Related Products

Product	Cat. No.	Size
Anti-HA Affinity Beads	SA068001	1 ml
	SA068005	5 ml
	SA068025	25 ml
	SA068100	100 ml
	SA068500	500 ml
	SA06801L	1 L