



Anti-DYKDDDDK Affinity Beads

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1. Product Description

Flag tag is a polypeptide consisting of eight hydrophilic amino acids located on the surface of the fusion protein, making it more easily to bind to antibodies and be digested by enterokinase. **Anti-DYKDDDDK Affinity Beads** consists of 90 µm beads of 4% agarose, to which anti-flag (DYKDDDDK) antibody has been coupled as affinity ligand. **Anti-DYKDDDDK Affinity Beads** can be used to purify flag tag fusion proteins expressed in prokaryotes, yeast or mammalian cells or for immunoprecipitation.

Table 1. Characteristics of **Anti-DYKDDDDK Affinity Beads**

Item	Description
Matrix	4% agarose
Ligand	Anti-DYKDDDDK mouse monoclonal antibody
Static Binding Capacity	>1mg DYKDDDDK fusion protein/ml medium
Particle size	45-165 µm
Maximum Pressure	0.1 MPa, 1 bar
Storage Solution	1×PBS containing 0.02% NaN ₃
Storage Temperature	2-8℃

2. Reagents Preparation

2.1 Buffer Preparation

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

Binding/Wash Buffer: 50 mM Tris, 0.15 M NaCl, pH 7.4

Acid Elution Buffer: 0.1 M Glycine HCl, pH 3.0

Competitive Elution Buffer: 50 mM Tris, 0.15 M NaCl, 100-500 µg 3*Flag peptide/ml, pH 7.4

Neutralization Buffer: 1 M Tris-HCl, pH 8.5

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 0.22 µm or 0.45 µm 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-DYKDDDDK 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 Elution:** 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 1×PBS containing 0.02% NaN₃ 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 minor filter the mixture to collect the beads.

4) Load the Beads into the chromatographic 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% NaN₃ 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-DYKDDDDK 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 Elution:** 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-DYKDDDDK antibody can also be denatured by SDS in the SDS-PAGE sample loading buffer. So **Anti-DYKDDDDK 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℃ 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. Chemical Compatibility

Table 2. Chemical compatibilities for **Anti-DYKDDDDK Affinity Beads**

Reagent	Concentration	Note
β- Mercaptoethanol	10mM	Avoid to use during Purification.
DTT	80mM	
SDS	--	
EDTA	5mM	High EDTA concentration may decrease the recovery rate of Protein.





(continued table)

Reagent	Concentration	Note
Tween-20	5%	High concentration will affect the binding efficiency of the tagged protein.
Triton X-100	5%	
NP-40	4%	
Guanidine HCl	0.3M	High concentration may denature the antibody ligand.
Urea	1.5M	
Glycerol	20%	High concentration will affect the binding of tagged protein.
NaCl	1M	Reduce nonspecific adsorption.

5. 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 in elution fraction.	Target protein is not stable.	Prepare new sample. Operate at low temperature. Add protease inhibitor in the sample solution.
	No DYKDDDDK fusion protein in the sample.	Detect the tag by Western Blot.
	Low expression of target protein.	Optimize the protein expression level. Increase the sample amount. Reduce the concentration of NaCl.
	The sample is not eluted completely.	Use 2-8 M 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.

6. Related Products

Product	Cat. No.	Size
Anti-DYKDDDDK Affinity Beads	SA042001	1 ml
	SA042005	5 ml
	SA042025	25 ml
	SA042100	100 ml
	SA042500	500 ml
	SA04201L	1 L

