Anti-DYKDDDDK Magarose Beads

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1. Product Description

Magarose Beads have superparamagnetism and rapid magnetic responsiveness. Peptides, proteins, antibodies, oligomeric nucleotide biological ligands can be coupled to the surface of microspheres. **Magarose Beads** is an important carrier of the medical and molecular biology research tools.

Flag tag is a polypeptide fragment consisting of eight hydrophilic amino acids, located on the surface of the fusion protein and therefore more likely to bind to antibodies and be broken down by enterokinases.**Anti-DYKDDDDK Magarose Beads** use anti-flag (DYKDDDDK) antibodies as affinity ligands.It can purify Flag-tag fusion protein expressed in prokaryotic, yeast or mammalian cells in one step.

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

Item	Description
Matrix spherical	Magnetic agarose
Ligand	Anti-DYKDDDDK mouse monoclonal antibody
Static binding capacity	>1mg DYKDDDK fusion protein/ml medium
Particle size (μm)	30-100
Beads concentration	20%(V/V) slurry
Storage solution	1X PBS containing 0.02% NaN_3
Storage temperature	2-8℃

2. Reagents Preparation

2.1 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.

2.2 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 ug 3×flag peptides/ml, pH 7.4

Neutralization Buffer: 1 M Tris-HCl, pH 8.0

3. Purification Procedure

3.1 Preparation of the Magnetic Beads

1) Completely resuspend the beads by shaking or vortexing the vial.
2) Transfer appropriate amount **Anti-DYKDDDDK Magarose Beads**(20% v/v) into a clean tube.
3) Place the tube on a magnetic separation rack to collect the beads. Remove and discard the supernatant.
4) Add 0.5 ml Binding/Wash Buffer to the tube and invert the tube several times to mix. Use the magnetic separation rack to collect the beads and discard the supernatant. Repeat this step twice.

3.2 Sample Adsorption

Add the sample solution to the magnetic bead pretreated in Step 3.1, swirl and mix evenly. Place the sample on a rotator at room temperature. After about 30 min, Use the magnetic separation rack to collect the beads and discard the supernatant.

3.3 Wash the miscellaneous

Add 5 column volumes Wash Buffer to the centrifuge tube,swirl and mix evenly, and place the centrifuge tube on the magnetic separation rack for 1minute to collect the beads. Remove and discard the supernatant. Repeat the washing for 2 times.

3.4 Elution of Target Protein

A Acid elution:

Add 3 to 5 column volumes of Acid Elution Buffer to the tube, mix on a rotator at room temperature for 5-10 minutes, place the centrifuge tube on the magnetic separation rack for 1minute to collect the beads. Collect the eluate. This operation can be repeated once.

Add a tenth of the volume of neutralizing solution to the eluate in advance and adjust the pH to 7.0-8.0.

Note: after acid elution, magnetic Beads should be balanced immediately with the Binding Buffer. The **Anti-DYKDDDDK Magarose Beads** should be no more than 20 min in the eluent.

B Competitive elution:

Add 3 to 5 column volumes of Competitive Elution Buffer to the tube and incubate at room temperature for 30min. Place the centrifuge tube on the magnetic separation rack for 1minute to collect the beads. Collect the eluate. This operation can be repeated once. Elution samples should be placed at 4℃ temporary and stored at -20℃ for a long time.

C Denaturing elution:

DTT and β-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 Magarose Beads** can not be reused again after denaturing elution.

Add 2X SDS-PAGE Sample Buffer with equal volume of magnetic beads into each tube and heat the tube at 100°C for five minutes. Place the centrifuge tube on the magnetic separation rack for 1minute to collect the beads. Take the supernatant for SDS-PAGE electrophoresis.

Note: **Anti-DYKDDDDK Magarose Beads** cannot be reused after denaturing elution.

4. Chemical Compatibility

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

Reagent	Tolerance levels	Note
β-mercaptoethanol	10mM	Use should be avoided in the purification process, if used in IP, the filler cannot be recycled and reused.
DTT	80mM	
SDS	--	
EDTA	5mM	High EDTA concentration may decrease the recovery rate of Protein.
Tween-20	5%	High concentration will affect the binding efficiency of the tagged protein.
Triton X-100	5%	
NP-40	4%	
Gua-HCl	0.3M	Too high a concentration denatures the antibody.
urea	1.5M	
glycerin	20%	High concentration will affect the binding of tagged protein.
Sodium chloride	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.

(continued table)

Problem	Possible Cause	Solution
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 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 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.

6. Related Products

Product	Cat. No.	Size
Anti-DYKDDDDK Magarose Beads	SM009001	1ml
	SM009005	5ml
	SM009025	25ml
	SM009100	100ml
	SM00901L	1L
Anti-DYKDDDDK Affinity Beads	SA042001	1 ml
	SA042005	5 ml
	SA042025	25 ml
	SA042100	100 ml
	SA042500	500 ml
	SA04201L	1 L