Anti-RFP Affinity Beads 4FF

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1. Product Description

The RFP (Red fluorecent protein) is a molecular marker isolated from anemone and is often used to study protein localization, reporter gene expression and protein-protein interaction. The agarose bead surface is coated with anti-RFP antibody, a highly specific mouse monoclonal antibody that can be used for immunoprecipitation, co-immunoprecipitation, affinity chromatography of natural RFP, RFP mutants and their fusion proteins. Characteristics of **Anti-RFP Affinity Beads 4FF**: 1) The ligand antibody has a very high affinity with RFP, which can efficiently capture RFP tagged proteins in the cell lysis; 2) After immunoprecipitation, SDS-PAGE test has a cleaner background and is more conducive to the identification of protein interactions. 3) Agarose material is characterized by low non-specific adsorption and good compatibility, with a capacity of more than 1 mg RFP fusion protein/ml (up to 5 mg/ml), and can be used for the massive purification of RFP fusion proteins.

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

Item	Description
Matrix Spherical	4% agarose
Ligand	Anti-RFP Antibody
Static Binding Capacity	>1mg RFP fusion protein/ml medium
Particle size	45-165 μm
Maximum Pressure	0.3 MPa, 3 bar
pH	3-10
Reagent tolerance	Stable up to 80℃, 1 mM DTT, 3 M Guanidinium•HCl, 8 M Urea, 2 M NaCl, 2% Nonidet P40 Substitute, 1% SDS, 1% Triton X-100
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 0.22 μm or 0.45 μm filter before use.

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

Elution Buffer: 0.1 M Glycine HCl, pH 3.0

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-RFP Affinity Beads 4FF.** 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 to the column to equilibrate the beads.
3) Load the sample to the column. 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 10-20 column volumes Wash Buffer or until the absorbance of the effluent at 280 nm is stable.
5) **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 20 min.
6) Regeneration with 3 column volumes of Elution Buffer. Equilibrate the column with 3 column volumes of Equilibration Buffer , 5 column volumes of distilled water. Finally store the resin with 1×PBS 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 30 min (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 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 Equilibration Buffer , 5 column volumes of distilled water. Finally store the resin with 1×PBS 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-RFP Affinity Beads 4FF** and add 40 µl (column volume 20 µl) to 1.5-2 ml micro centrifuge tube. Centrifuge for 1 min at 5000×g and discard supernatant.
2) Add 500 µl of Binding Buffer, centrifuge for 1 min at 5000×g and discard supernatant. Repeat this step two times.
3) Add 200-1000 µl 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.
4) Centrifuge for 1 min 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 1 min at 5000×g and discard supernatant. Repeat this step two times.
A: Acid Elution: Elute the target protein with 100 µl Elution Buffer. Incubate the tube at room temperature with mixing (on a shaker or rotator) for 5min, centrifuge for 1 min at 5000×g. Collect the supernatant and add a tenth of the volume of neutralizing solution to the eluate in advance.
B: Degeneration Elution: Add 20 µl 2×SDS-PAGE Sample Buffer to the tube and mix well. Heat the tube at 100℃ for five minutes. Centrifuge for 1 min at 5000×g and transfer the supernatant containing desired sample into a new tube.
6)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 RFP fusion protein in the sample.	Detect the RFP 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 2-8 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.

5. Related Products

Product	Cat. No.	Size
Anti-RFP Affinity Beads 4FF	SA072001	1 ml
	SA072005	5 ml
	SA072025	25 ml
	SA072100	100 ml
	SA072500	500 ml
	SA07201L	1 L