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# Anti-YFP Affinity Beads 4FF

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## 1. Product Description

The fluorecent protein is often used to study protein localization, reporter gene expression and protein-protein interaction. YFP (yellow fluorescent protein), a genetic mutant of green Fluorescent Protein (GFP), migrates its fluorescence to the red spectrum mainly because the threonine at position 203 of GFP is replaced by tyrosine. The yellow fluorescent protein fusion protein can spontaneously fluoresce. The localization of the target gene in the cell can be detected without antibody hybridization of the target protein, with little interference from other substances. The agarose can be used for immunoprecipitation, co-immunoprecipitation, affinity chromatography of natural YFP and their fusion proteins. **Anti-YFP Affinity Beads 4FF** can also detect and purify GFP, EGFP and their fusion proteins . It cannot bind BFP fusion protein.

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

| Item | Description |
|---|---|
| Matrix Spherical | 4% agarose |
| Ligand | Anti-YFP Antibody |
| Static Binding Capacity | >1mg YFP 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-YFP 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-YFP Affinity Beads 4FF** and add 40μl (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 5 min, 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.<br>Operate at low temperature.<br>Add protease inhibitor in the sample solution. |

| | | |
|---|---|---|
| | No YFP fusion protein in the sample. | Detect the YFP tag by Western Blot. |
| | Low expression of target protein. | Optimize the protein expression level.<br>Increase the sample amout.<br>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.<br>Increase the concentration of NaCl in the Wasing Buffer. |

## 5. Related Products

| Product | Cat. No. | Size |
|---|---|---|
| Anti-YFP Affinity Beads 4FF | SA075001 | 1 ml |
| | SA075005 | 5 ml |
| | SA075025 | 25 ml |
| | SA075100 | 100 ml |
| | SA075500 | 500 ml |
| | SA07501L | 1 L |