

# Anti-RFP Affinity Gel

## 1 Packing list

Components	HY-K0233-1 mL	HY-K0233-5 mL	HY-K0233-10 mL
Anti-RFP Affinity Gel	1 mL	5 mL	10 mL

## 2 Introduction

The Red Fluorescent Protein (RFP) is derived from *Discosoma* sp. Its red fluorescence exhibits strong penetration, making it particularly useful for imaging and cell tracking studies. RFP has been extensively utilized as a reporter gene in the fields of cell biology and molecular biology.

MCE Anti-RFP Affinity Gel is produced through the covalent coupling of high-quality RFP antibody with agarose. It boasts a high loading capacity, exceptional specificity, and stability. This gel can be used for the detection and purification of native RFP, RFP mutants, and their fusion-expressed proteins. Moreover, it can be utilized for immunoprecipitation (IP) assays.

The product contains 0.5 mL of gel per 1 mL of total volume. Prior to use, ensure the gel is thoroughly resuspended and mixed before aspiration.

## 3 Characteristics

### Information

Composition	4% cross-linked agarose
Ligand	Anti-GFP Antibody
Binding Capacity	>1 mg GFP tagged protein/mL of Resin
Bead Diameter	45-165 $\mu$ m
Pressure	0.3 MPa, 3 bar
Compatible	Stable up to 80°C, 1 mM DTT, 3 M Guanidinium-HCl, 8 M Urea, 2 M NaCl, 2% NP-40, 1% SDS, 1% Triton X-100
Storage Solution	1× PBS, 0.02% NaN <sub>3</sub>

## 4 General Protocol

### Buffer Preparation

Binding/Washing Buffer	50 mM Tris, 0.15 M NaCl, pH 7.4
Elution Buffer	0.1 M Glycine, pH 3.0
Neutralization buffer	1 M Tris-HCl, pH 8.0
Storage Buffer	PBS, 0.02% (w/v) NaN <sub>3</sub>

Note: It is recommended to prepare all buffers with ultrapure water and. After preparation, filter them through a 0.45  $\mu$ m or 0.22  $\mu$ m membrane for sterilization.

## Protein purification

It is recommended to filter the sample with a 0.22 µm or 0.45 µm filter before purification.

### Medium-pressure chromatography

1. Load the column: Load Anti-RFP Affinity Gel in an appropriate chromatography column and connect it to the chromatography system.
2. Equilibration: Equilibrate the column by washing it with 5× column volume of Binding Buffer. Repeat 2-3 times.
3. Loading: Loading sample using a pump or sample loops, collecting the effluent. Repeating this loading process can improve the binding efficiency.  
Note: a. Please choose a suitable gel volume according to the amount of protein to avoid exceeding the loading capacity of agarose.  
b. An increase in sample viscosity or volume may lead to column backpressure.
4. Washing: Wash the column with approximately 15-30× column volume of Washing Buffer to remove nonspecifically adsorbed hetero-proteins and collect the wash effluent until the absorbance at 280 nm stabilizes.
5. Elution: Elute the column with approximately 3-5× column volume of Elution Buffer, collect the eluent and immediately neutralize its pH with Neutralization Buffer (1/10 volume of total eluent volume), the collected eluates contain the target protein.  
Note: The eluates can be used immediately if stored at 4°C, or stored at -20°C for long term storage.
6. Regeneration: Thoroughly elute the column with 5× column volume of Elution Buffer. Equilibrate the column with Washing Buffer.  
Note: The agarose should be balanced with Washing Buffer immediately after acidic elution, and the agarose should not be kept in the Elution Buffer for more than 20 min.
7. Storage: Equilibrate the resin with 5-10× column volume of Storage Buffer, disconnected the column and store at 2-8°C.

### Gravity column method

1. Load the column: Choose an appropriate volume of Anti-RFP Affinity Gel suspension according to the amount of samples and load it into the gravity chromatography column, eliminating the protective solution.
2. Equilibration: Equilibrate the column: Equilibrate the resin with 5× column volume of Binding Buffer, repeat 2-3 times.
3. Loading: Loading sample and collect the effluent. It is recommended that the samples maintain contact with the agarose at least 2 min.  
Note: Repeated loading can improve the binding efficiency.
4. Washing: Wash resin with 10-15× column volume of Washing Buffer to eliminate non-specifically adsorbed hetero-proteins, collect the effluent.
5. Elution: Elute the column with approximately 3-5× column volume of Elution Buffer, collect the eluent and immediately neutralize its pH with Neutralization Buffer (1/10 volume of total eluent volume), the collected eluates contain the target protein.  
Note: The eluates can be used immediately if stored at 4°C, or stored at -20°C for long term storage.
6. Regeneration: Thoroughly elute the column with 5-10× column volume of Elution Buffer. Equilibrate the column with Washing Buffer.  
Note: The agarose should be balanced with Washing Buffer immediately after acidic elution and the agarose should not be kept in the Elution Buffer for more than 20 min.
7. Storage: Wash the column with 5× the volume of Storage Buffer and store at 2-8°C.

### Centrifugation

1. Preparation of Agarose: Choose an appropriate volume of Anti-RFP Affinity Gel suspension according to the amount of samples and transfer it into a tube, centrifuge at 5,000 g for 1 min and discard. Wash the resin with 5× the volume of Washing Buffer, centrifuge at 5,000 g for 1 min and discard the supernatant. Repeat 2-3 times.
2. Binding: Add the sample and incubate at 4°C for 2-4 h (or 37°C for 0.5-2 h).
3. Washing: Centrifuge at 5,000 g for 1 min and discard the supernatant (e.g., if required, save supernatant for subsequent analysis). Wash the resin with 5× the volume of Washing Buffer, centrifuge at 5,000 g for 1 min and discard the supernatant. Repeat 3-5 times.
4. Elution: Elute the column with approximately 3-5× column volume of Elution Buffer, incubate at room temperature for 5-10 minutes, centrifuge at 5,000 g for 1 min. The collected supernatant contains the target protein. The final collected eluate is acidic, Neutralization Buffer should be added immediately to adjust the pH (1/10 volume of total eluent volume), then the samples can be used for functional analysis. Repeat 2-3 times and collect the supernatant each time.  
Note: a. The agarose should be balanced with Washing Buffer immediately after acidic elution, and the agarose should not be kept in the Elution Buffer for more than 20 min.  
b. The eluates can be used immediately if stored at 4°C, or stored at -20°C for long term storage.
5. Regeneration and storage: Wash the resin with 5-10× the volume of Washing Buffer, then wash the resin with 5-10× the volume of ddH<sub>2</sub>O. Finally, wash the resin with 2× the volume of Storage Buffer and store at 2-8°C.

## IP

### 1. Preparation of Agarose

- 1) Add 40  $\mu$ L of Anti-RFP Affinity Gel suspension (approximately 20  $\mu$ L of gel) to a 1.5 mL tube, centrifuge at 5,000 g for 1 min and discard the supernatant.
- 2) Add 500  $\mu$ L of Binding Buffer and mix well, centrifuge at 5,000 g for 1 min and discard the supernatant. Repeat 3-4 times.

### 2. Sample Binding

- 1) Add 200-1,000  $\mu$ L of sample and mix thoroughly, gently rotate the tube at 4°C for 2 hours. If you want to enhance the binding efficiency, you can incubate it overnight.

Note: For proteins prone to degradation, it is recommended to add protease inhibitors.

- 2) Centrifuge at 5,000 g for 1 min transfer the supernatant to a new centrifuge tube (the supernatant can be used to detect any residual RFP-tagged protein).

### 3. Washing

Add 1 mL Washing Buffer to the agarose and mix thoroughly, centrifuge at 5,000 g for 1 min and discard the supernatant. Repeat 2-3 times until the OD<sub>280</sub> of the supernatant liquid is < 0.05.

### 4. Elution

Two recommended elution methods depend on protein characteristics or further usage.

- 1) Acidic condition Elution with Elution Buffer: This method maintains the original biological activity of the eluted samples and is suitable for subsequent functional analyses.

Add 50-100  $\mu$ L of Elution Buffer to the tube. Mix well and incubate for 5-10 min at room temperature, centrifuge at 5,000 g for 1 min and transfer the supernatant to a new tube. The final collected eluate is acidic, Neutralization Buffer should be added immediately to adjust the pH (1/10 volume of total eluent volume), then the samples can be used for functional analysis.

Note: The eluates can be used immediately if stored at 4°C, or stored at -20°C for long term storage.

- 2) Gel Electrophoresis and Immunoblotting Elution with SDS-PAGE Loading Buffer.

Add 20-50  $\mu$ L of 2× SDS-PAGE Loading Buffer to the tube. Mix well and heat for 5 min at 95°C. Centrifuge at 5000 g for 1 min. Reserve the supernatant for SDS-PAGE analysis.

Note: As regular SDS-PAGE Loading Buffer contains  $\beta$ -mercaptoethanol and DTT, which can break the light and heavy chains of the antibody in the packing. And the SDS-containing Loading Buffer can denature the medium ligand. Anti-RFP Affinity Gel cannot be reused after denaturing elution.

## 5 Storage

4°C, 2 years

Do not freeze

## 6 Precautions

1. Please resuspend the gel thoroughly before use.
2. Before using this product for IP (Immunoprecipitation) experiments, it is necessary to confirm the expression status of RFP tagged proteins in the sample.
3. To minimize protein degradation, protease inhibitor cocktails (MCE Cat. No. HY-K0010, HY-K0011) are highly recommended.
4. Do not use cell lysate samples containing DTT. DTT may cause the RFP antibodies on the gel to detach.
5. This product is for R&D use only, not for drug, household, or other uses.
6. For your safety and health, please wear a lab coat and disposable gloves to operate.