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Anti-Flag Affinity Gel

1 Contents

Components	HY-K0217-1 mL	HY-K0217-5 mL	HY-K0217-10 mL
Anti-Flag Affinity Gel (Settled Gel)	1 mL	5 mL	10 mL

2 Introduction

MCE Anti-Flag Affinity Gel is a purified mouse IgG2b monoclonal antibody covalently attached to agarose Sepharose 4B. With high protein-binding capacity (>1.1 mg protein/mL) and stability, this product is ideal for high performance purification or immunoprecipitation (IP) of Flag-tagged proteins expressed in *E.coli*, yeast, insect and mammalian expression systems.

3 Characteristics

Isotype	Mouse IgG2b
Application	Protein Purification, IP, Co-IP
Binding Capacity	Minimum 1.1 mg of Flag-tagged proteins per mL of gel
Volume	5 µL gel for 500 µL lysate
Storage Buffer	50% glycerol, 10 mM Na_3PO_4, 150 mM NaCl, 0.02% (w/v) sodium azide, pH 7.4

4 General Protocol

Recommended Buffer

Wash Buffer	TBST: 50 mM Tris-HCl, 150 mM NaCl, 0.5% Tween-20, pH 7.4
Elution Buffer A	150 mM Glycine, pH 2.5-3.1
Elution Buffer B	1 mg/mL 3× Flag Peptide, 50 mM Tris, 150 mM NaCl, pH 7.4
Neutralization Buffer	1 M Tris-HCl, pH 8.0

1. Preparation of Anti-Flag Affinity Gel

1.1. Thoroughly suspend the Anti-Flag Affinity Gel. Transfer 10 µL of the gel suspension (about 5 µL of settled gel) to a clean tube.

1.2. Add 600 µL of Wash Buffer to resuspend the gel. Centrifuge at 10,000 rpm for 30 seconds. Remove the supernatant carefully. Be sure that most of the wash buffer is removed and no gel is discarded. Repeat 3-4 times.

2. Protein Binding

2.1. Add 500 µL of cell lysate (the sample containing Flag-tagged protein) to the cleaned gel from step 1.2. Incubate for 2 hours at 4°C while gently rotating the tube. For higher binding efficiency, please incubate overnight.

2.2. Centrifuge at 10,000 rpm for 30 seconds, and transfer the supernatant to a new cube (the supernatant can be used to determine whether there is residual Flag-tagged protein).

3. Washing

Wash the gel with 500 μL of Wash Buffer, centrifuge at 10,000 rpm for 30 seconds. Remove the supernatant carefully. Be sure that most of the wash buffer is removed and no gel is discarded. Wash until the OD_{280} of the supernatant liquid＜0.05.

4. Elution/Detection

Three elution methods are recommended according to protein characteristics or further usage:

4.1 Elution with SDS-PAGE Loading Buffer for gel electrophoresis and immuoblotting.

Add 50 μL of 1× SDS-PAGE Loading Buffer to each tube. Mix well and boil for 5 minutes. Centrifuge at 10,000 rpm for 30 seconds. Keep the supernatant for SDS-PAGE analysis.

4.2 Elution with Elution Buffer A under acidic condition.

Add 50 μL of Elution Buffer A to each tube. Mix well and incubate for 10 minutes at room temperature. Centrifuge at 10,000 rpm for 30 seconds and transfer the supernatant to a new tube. Add 25 μL of Neutralization Buffer for each 50 μL of eluate to neutralize the low pH, which may help preserve bioactivity of target proteins.

4.3 Elution with Elution Buffer B under native condition.

Add 30-50 μL of Elution Buffer B to each tube. Incubate with gentle shaking or on a rotator for 1 hour at room temperature or 2 hours at 4ºC. Centrifuge at 10,000 rpm for 30 seconds and transfer the supernatant to a new tube.

For immediate use, store the eluates at 4ºC, or store at -20ºC for long term storage.

5 Storage

Store at -20°C, and is stable for at least 2 years.

6 Precautions

1. Please resuspend the gel thoroughly before use.
2. For the best results, determine optimal conditions for expression of Flag-tagged fusion protein before attempting immunoprecipitation.
3. To minimize protein degradation, protease inhibitor cocktails (MCE Cat. No.: HY-K0010, HY-K0011) are highly recommended.
4. Do not use cell lysate containing dithiothreitol (DTT). DTT may cause the Anti-Flag antibody to leach from the gel.
5. This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.
6. For your safety and health, please wear a lab coat and disposable gloves to operate.

7 Troubleshooting

Problem	Possible Cause	Solution
High background	Nonspecific binding of protein to the antibody, gel or EP tubes	Pre-clear lysate to remove nonspecific binding proteins
		After suspending gels for the final wash, transfer the entire sample to a clear EP tube
	Washing times were not sufficient	Increase the number and time of washes
Little or no Flag-tagged protein is detected	No or minimal tagged protein was expressed	Verify protein expression by SDS-PAGE or Western Blot by using a Flag-tagged positive control
		Increase the amount of lysate used for IP
	Flag-tagged protein was degraded	Prepare fresh lysate
		Use appropriate protease inhibitors (MCE Cat. No.: HY-K0010, HY-K0011)
	Incubation time was inadequate	Prolong the incubation time
	Interfering substance was contained	Do not use cell lysate containing dithiothreitol (DTT), 2-mercaptoethanol, or other reducing agents
		Excessive detergent concentration may interfere with the antibody-antigen interaction