Ver. HB230704

Anti-DYKDDDDK (Flag) Affinity Gel

Product description

Anti-DYKDDDDK (Flag) Affinity Gel is prepared by conjugating high quality murine IgG2b monoclonal antibody with 4% highly cross-linked agarose gel. This product has a high Flag label fusion protein loading capacity (at least 1.1 mg protein/mL gel) and less non-specific binding of miscellaneous proteins. It can be used for immunoprecipitation (IP) and purification of fusion proteins with Flag labels.

Anti-DYKDDDDK (FLAG) Affinity Gel can bind to the Met modified N-flag fusion Protein (MET-flag-Protein), N-flag fusion protein (Flag-protein), C-terminal FLAG Fusion Protein (protein-flag).

Components

Components No.	Name	20584ES03	20584ES08	20584ES25	20584ES60
20584	Anti-DYKDDDDK (Flag) Affinity Gel	1 mL	5 mL	25 mL	100 mL

Note: In order to facilitate customers to use 1×Flag polypeptide elution, this product package is accompanied by 1×Flag polypeptide (Cat#20572ES).

Based on the different specifications of 1×Flag polypeptide (Cat#20572ES), 1 mL gel package is combined with 5 mg peptide, and other gel specifications are combined with 1 mL: 1 mg ratio of 5 mg specification 1×Flag polypeptide (Cat#20572ES).

Specifications

Clone	1A3
Isotype	Mouse IgG2b
Application	Protein purification、IP
Protein binding capacity	⩾1.1 mg protein/mL Gel
Storage buffer	TBS，50% glycerin，pH7.4，contains 0.02% (w/v) sodium azide

Shipping and Storage

The products are shipped with ice pack, and unopened products can be stably stored at -25~-15°C for 1 year. **Do not freeze in solution without glycerin!**

Instructions

1. Preparation of cell lysate (Taking mammalian cells as an example)

Note: The sample should not contain insoluble particles, which can be filtered by 0.22 μm filter membrane or centrifuged by 10,000×g for 15 min. Protease inhibitors can be added if necessary; If the sample is too sticky, it can be treated with nuclease.

Cell density 70-90%, 100 mm petri dish (about 10^6-10^7 cells), adding 1mL lysate (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1%Triton X-100). A protease inhibitor Cocktail (Cat#20123ES) was recommended.

1) Wash the cells

Adherent cells: Growth medium was removed, washed twice with PBS, PBS was removed, and lysate (10^6-10^7 cells/mL) was added.

Suspension cells: The cells were transferred to a centrifuge tube, centrifuged at 420×g for 5 min, and the supernatant was removed. Re-suspension was performed with PBS, centrifuged at 420×g for 5 min, repeated once, supernatant was removed, and lysis solution was added for re-suspension (10^6-10^7 cells/mL).

2) After adding the lysate, incubate for 15-30 min.

3) Centrifugation, 12,000×g, 10 min. Scrape off the adherent cells before centrifugation.

4) Collect the supernatant into the pre-cooled sample tube. Supernatant can be stored at -70°C.

2. Application

The target protein can be purified using chromatographic columns or immunoprecipitation (IP). About 100 mL of cell lysate can be purified by 1-3 mL gel chromatography column. If the sample volume is small (1-2 mL of cell lysate), immunoprecipitation (IP) can be used. Solution systems are recommended for handling large volumes of cell lysate.

2.1 Chromatographic column method

2.1.1 Column Installation

1) Prepare the empty chromatographic column (Cat#20520ES-20526ES); Wash the column twice with TBS (50 mM Tris-HCl, pH 7.4,150 mM NaCl) or other suitable buffer solution that is 2-3 times the column volume.

2) Reverse mix Anti-Flag affinity purification gel to ensure gel uniformity. Take 1-3 mL gel packed column (purified about 100 mL cell lysate).

3) Wash twice with TBS of 2-3 times the column volume. After the liquid runs out, rinse with 0.1 M Gly-HCl,pH 2.7-3.0 of 3 times the volume to ensure the smooth gel surface. Gly-HCl buffer treatment time should not exceed 20 min.

4) Balance the gel column with 10 times the volume of TBS until the effluent reaches a neutral pH.

2.1.2 Purification

1) Loading(one of the following two methods can be selected) :

A. Fill the chromatographic column with protein cracking solution and repeat loading 2-3 times to increase the binding amount of protein and gel as much as possible.

B. Protein lysate was added into the TBS balanced gel and incubated at 28°C for 60-90 min. The gel was suspended to increase the binding amount of protein and gel.

2) Cleaning: Cleaning with 10-20 times the column volume of TBS to remove the specific binding proteins.

3) Elution (one of the following three methods can be selected) :

Different proteins have slightly different optimal elution methods, and 1 × Flag polypeptide (Cat#20572ES) is preferred

A. Alkaline elution: Eluting with 1 times column volume of alkaline eluent (0.1 M Tris, 0.5 M NaCl, pH 12), immediately after elution adding 1 M HCl with 1/10 elution volume to neutralize elution sample, repeat 5 times. Elution time does not exceed 20 min.

B. Acid elution: 1 M Tris (pH 8.5) of 1/10 column volume, , was added to the collection tube, eluted with 1 times column volume of 0.1 M Gly-HCl, pH 2.7-3.0, repeat 5 times. Elution time does not exceed 20 min.

C. 1×Flag polypeptide (Cat#20572ES) elution: Flag polypeptide solution (100-500 μg/mL) prepared with TBS was eluted, and eluted with 1 times column volume polypeptide solution, repeat 5 times.

2.1.3 Regeneration and preservation of filler

1) Column regeneration: regeneration immediately after use, clean with 0.1 M Gly-HCl,pH 2.7-3.0(3 times column volume), immediately balance with TBS until neutral pH is reached.

2) Preservation: Clean with 10 times column volume of TBS (containing 50% glycerin and 0.02% sodium azide), then add 5 mL of the solution to the column, and store at 2-8°C or -20°C.

2.2 Immunoprecipitation (IP)

2.2.1 Immunoprecipitation (IP)

1) Fully re-suspension of Anti-Flag Affinity Gel to form a uniform solution as far as possible. Put 40 µL of the mixture (20 µL gel) into the new centrifuge tube.

2) Centrifuge at 5,000-8,200×g for 30 s to precipitate the gel at the bottom of the centrifuge tube, and let it stand for 1-2 min before adding the sample. Remove supernatant, carefully at this point to avoid absorbing gel.

3) Add 500 µL TBS, gently reinsert the Anti-Flag Affinity Gel, centrifuge at 10,000 rpm for 30 s, discard the supernatant, and repeat the above steps once.

4) Optional steps. In order to remove the unbound antibodies in the gel, 500µL 0.1M glycine HCl, pH 2.7-3.0 was added to clean the gel, and supernatant was removed by centrifugation. TBS of 3 times the volume was added. Then Shake gently for 2-3 min and centrifuge at 5,000-8,200×g for 30 s. The supernatant was discarded, and the pH of the last clear was neutral after repeated washing. The treatment time of Glycine HCl should not exceed 20 min.

5) Add 200-1000 µL cell lysate, and adjust the lysate to the final volume of 1 mL if necessary. The volume of cell lysate depends on the expression of Flag fusion protein. For positive control, 1 mL TBS and 4 µL 50 ng/µL Flag-BAP fusion protein (about 200 ng) were added. For negative control, just add 1 mL cleavage buffer (without protein).

6) Incubate slowly at 4°C for 2 hours. If you need to improve the binding efficiency, can be extended to overnight.

7) Centrifuge at 5,000-8,200×g for 30 s to remove supernatant.

8) The above precipitation was lightly mixed with 0.5mL TBS, centrifugated at 5,000-8,200×g for 30 s to exhaust the supernatant, repeat 3 times.

2.2.2 Elution of Flag Fusion Protein (one of the following four methods can be selected)

Different proteins have slightly different optimal elution methods, and 1 × Flag polypeptide (Cat#20572ES) is preferred

1) Alkaline elution (0.1 M Tris，0.5 M NaCl，pH 12)

Add 100µL alkaline eluent, incubate at room temperature for 10 min (slowly shaking), centrifuge at 5,000-8,200×g for 30 s, and transfer the supernatant to a new tube (containing 10 µL 1 M HCl). Be careful not to inhale the gel. If used immediately, the supernatant can be stored at 4°C, -20°C for a long time.

2) Acid elution (0.1M Gly-HCl, pH 2.7-3.0)

Add 100 µL 0.1 M Gly-HCl, pH 2.7-3.0, incubate at room temperature for 10 min (slowly shaking), centrifuge at 5,000-8,200×g for 30 s, transfer the supernatant to a new tube (containing 10 µL 0.5 M Tris-HCl, pH 8.5, 1.5 M NaCl). Be careful not to inhale the gel. If used immediately, the supernatant can be stored at 4°C, -20°C for a long time.

3) 1×Flag polypeptide (Cat#20572ES) elution

Add 100 µL 1 × Flag polypeptide eluent (100-500 µg/mL), incubate at room temperature for 30 min (slowly shaking), centrifuge 5,000-8,200×g for 30 s, and transfer the supernatant to the new tube (do not inhale the gel). If used immediately, the supernatant can be stored at 4°C, -20°C for a long time.

4) SDS-PAGE loading buffer elution

Add 20 μL 2× loading buffer (125 mM Tris-HCl, pH 6.8, 4% SDS, 20% glycerin, 0.004% bromophenol blue) , boiled for 5 min, and centrifugated at 5,000-8,200×g for 30s. Supernatant can be directly SDS-PAGE electrophoresis, or WB detection.

2.2.3 Regeneration and preservation of filler

1) Regeneration: regeneration immediately after use, add 0.1M Gly-HCl, pH2.7-3.0(3 times column volume), shake gently for 3-5 min, centrifuge at 5,000-8,200×g for 30 s, discard the supernatant. Immediately balance with TBS: add 3 times gel volume TBS, gently shake for 2-3 min, centrifuge at 5,000-8,200×g for 30 s, collect supernatant to determine pH, if neutral, then proceed to the next step, if still acidic, repeat the balance step.

2) Preservation: Clean with 10 times gel volume TBS (containing 50% glycerin and 0.02% sodium azide), then add appropriate amount of the preservation solution to the filler, and store at 2-8°C or -20°C. The amount of buffer added can be restored to before purification. The volume ratio of glue: buffer =1:1, which can increase the volume of buffer liquid. The buffer is mainly to provide the necessary pH value and avoid freezing at -20°C.

3. Solution system

1) Adjusting the pH of protein lysate to pH 7-8, containing 0.15M salt ions, such as NaCl, can reduce non-specific protein binding. The insoluble components of Flag fusion protein lysate should be removed.

2) The Anti-Flag affinity purification gel was re-suspended and transferred to a new centrifuge tube. 3 times the volume of TBS, gently shake for 2-3 min, centrifuge at 5,000-8,200×g for 30 s, discard the supernatant. Repeat washing until the pH is neutral.

3) The Flag fusion protein lysate was incubated with Anti-Flag affinity purification gel for 1 h, and shook gently during incubation to ensure full contact between protein and gel. The incubation time can be adjusted according to the situation.

4) Centrifuge at 5,000-8,200×g for 5 min to collect gel-Flag fusion protein;

5) Wash the gel with 10-20 times the column volume of TBS to remove specific proteins.

6) Eluting Flag fusion protein [refer to the above elution steps].

7) Regeneration and preservation of filler [refer to the above steps].

Notes

1.During acid elution, the treatment time of Gly-HCl buffer solution should not exceed 20 min.

2. Do not add reducing agents (such as DTT) to the loading buffer, as reducing agents will destroy antibodies.

3. SDS in the loading buffer will destroy antibodies, so Anti-Flag affinity purification gel cannot be reused.

4. For protein purification, it is recommended to pack the gel into a chromatographic column where all the solution can flow directly through. If in the tube, need to repeatedly centrifuge to remove the solution, the operation is tedious.

5.Except for samples and cracking solution, other solutions are recommended to be 3 times the volume and repeated 3 times. On the basis of ensuring the purification effect, the number of repeats can be reduced appropriately.

6.Both positive and negative control groups are recommended.

7.For your safety and health, please wear lab coats and disposable gloves for operation.

8.For research use only.