Pulldown™ Anti-GST Magnetic IP Kit

Cat #: D-AKE2034

Size: 20 T / 100 T

Storage: Store according to the recommended storage conditions of each component, stable for 12 months

Assay Principle

Anti-Glutathione S-transferase-Tag (GST-Tag) Magnetic Beads are prepared by covalently coupling Anti-GST-Tag Mouse Monoclonal Antibody to crosslinked Magnetic Beads, useful for detection and capture of fusion proteins containing a GST-Tag peptide sequence by commonly used immunoprecipitation procedures. The Optimized Anti-GST Magnetic Beads have more efficient antigen binding capacity. According to the structure, biological function and subsequent application requirements of the target protein, this kit provides two elution methods, including acid elution and denatured elution. Especially by using acid elution will not contain heavy chain and light chain of antibody, which could effectively avoid the interference of heavy chain and light chain of antibody in Western Blotting experiment after immunoprecipitation.

Materials Supplied and Storage Conditions

<table>
<tr><th colspan="2" rowspan="2">Kit components</th><th colspan="2">Size</th><th rowspan="2">Storage conditions</th></tr>
<tr><th>20 T</th><th>100 T</th></tr>
<tr><td rowspan="6">Part 1 of 2</td><td>Non-Denaturing Lysis Buffer</td><td>20 mL</td><td>100 mL</td><td>4°C</td></tr>
<tr><td>TBS (10×)</td><td>20 mL</td><td>100 mL</td><td>4°C</td></tr>
<tr><td>Anti-GST Magnetic Beads</td><td>0.4 mL</td><td>2 mL</td><td>4°C, Avoid freeze</td></tr>
<tr><td>Mouse IgG Magnetic Beads</td><td>80 μL</td><td>400 μL</td><td>4°C, Avoid freeze</td></tr>
<tr><td>Elution Buffer</td><td>2 mL</td><td>10 mL</td><td>4°C</td></tr>
<tr><td>Neutralization Buffer</td><td>0.2 mL</td><td>1 mL</td><td>4°C</td></tr>
<tr><td>Part 2 of 2</td><td>SDS-PAGE Loading Buffer (5×)</td><td>1 mL</td><td>2 mL×2</td><td>-20°C</td></tr>
</table>

Materials Required but Not Supplied

·Magnetic Separation Rack

·Vertical rotating mixer

·Freezing Centrifuge

·Precision Pipettes, Disposable Pipette Tips

·Deionized Water

·PBS Buffer

·Dounce homogenizer (for tissues)

Reagent Preparation

Non-Denaturing Lysis Buffer: Native protein lysis buffer, extract protein for IP samples. Ready to use as supplied. Place it on ice for use. Store at 4°C.

1×TBS: Add Deionized Water to the 10×TBS and dilute the 10×TBS to 1×TBS before use. Store at 4°C.

Anti-GST Magnetic Beads: Ready to use as supplied. Store at 4°C, Avoid frozen.

Mouse IgG Magnetic Beads: Ready to use as supplied. Store at 4°C, Avoid frozen.

Elution Buffer: Ready to use as supplied. Store at 4°C. Used for acid elution of non-denatured proteins.

Neutralization Buffer: Ready to use as supplied. Store at 4°C. Used for neutralize acid elution of non-denatured proteins.

SDS-PAGE Loading Buffer (5×): Ready to use as supplied. Store at -20°C.

Note: (1) Protease inhibitors are not necessarily added, it is recommended that different types of protease inhibitors should be added to the Non-Denaturing Lysis Buffer according to the experimental requirements; (2) It is recommended to use the centrifugal tube with low adsorption for experiment, which can reduce the adhesion of magnetic beads to the centrifugal tube wall. Adding 0.01%-0.1%(V/V) non-ionic detergent (such as Triton X-100, Tween-20 or NP-40) to 1×TBS can also effectively reduce the adhesion of centrifugal tubes to magnetic beads.

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Assay Procedure

A.Preparation of protein samples

Note: Prepare a certain amount of sample proteins, which were used as whole cell lysate (WCL) for subsequent Western Blotting detection.

1.Extract protein for Cell Samples:

(1)Collect cells (Adherent cells: 80% to 90% of monolayer cells were grown in a 10 cm cell culture dish. Remove the medium and wash with PBS once; Suspended cells: Collect 5×106 cells by centrifugation and washed by PBS once.

(2)Add 0.5-1 mL ice-cold Non-Denaturing Lysis Buffer to cells, lytic cells at 4°C for 5 min. During the process, the pipette is used to blow the mixture repeatedly, transfer cell suspension to a new tube.

(3)Centrifuge at 12,000 rpm for 10 min at 4°C, collect supernatant.

2.Extract protein for Tissue Samples:

(1)Tissue Samples: Weigh 0.1 g of tissue and add 1 mL Non-Denaturing Lysis Buffer, Homogenize tissue with Dounce homogenizer. (If the protein concentration is low, reduce the volume of Non-Denaturing Lysis Buffer).

(2)Transfer the homogenate to a new tube， lytic samples at 4°C for 5 min.

(3)Centrifuge at 12,000 rpm for 10 min at 4°C, collect supernatant.

3.Extract protein for Bacteria Samples:

(1)Collect bacteria by centrifugation (1,2000 rpm for 2 min at 4°C) and washed by PBS once.

(2)Per mL bacteria add 100-200 µL Non-Denaturing Lysis Buffer, Ultrasonic break in ice 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times).

(3)Centrifuge at 12,000 rpm for 10 min at 4°C, collect supernatant.

Note: (1) The sample must contain GST-tag protein and its complex; (2) For Immunoprecipitation, fresh samples are preferred; (3) In Immunoprecipitation experiments, the affinity between different antigen and antibody is different, and the binding of antigen to antibody is also affected by lysis buffer and wash buffer. If the Non-Denaturing Lysis Buffer does not provide the best experimental results, it is recommended to optimize the operation details or screen and prepare suitable by lysis buffer and wash buffer for experiment

B.Preparation of magnetic beads

Note: Per 500 µL of protein sample add 20 µL Anti-GST Magnetic Beads. Perform the following procedures, according to

add 20 μL Anti-GST Magnetic Beads.

(1)Add Anti-GST Magnetic Beads to a 1.5 mL centrifuge tube. Place the centrifuge tube on a Magnetic Separation Rack, let stand for 10 s, remove the supernatant.

(2)Add 1 mL 1×TBS to re-suspend Anti-GST Magnetic Beads, place the tube on a Magnetic Separation Rack, let stand for 10 s, remove the supernatant, repeat 3 times. Add 20 μL1×TBS to re-suspend Anti-GST Magnetic Beads.

C.Immunoprecipitation

(1)Add 500 μL protein samples to the processed Anti-GST Magnetic Beads, and incubate at room temperature for 1-2 h or overnight at 4°C (It is recommended to use vertical rotating mixer with Low-speed rotation).

Note: a) It is recommended to add Mouse IgG Magnetic Beads for immunoprecipitation in some samples as negative control, which could exclude the non-specific binding of IgG to the target protein or other specific biological molecules; b) In the case of very high background after immunoprecipitation using Anti-GST Magnetic Beads, it is recommended that Mouse IgG Magnetic Beads be used to preprocess samples to eliminate non-specific adsorption, then use Anti-GST Magnetic Beads for immunoprecipitation.

(2) Place the tube on a Magnetic Separation Rack, let stand for 10 s, remove the supernatant. **The supernatant can be transferred to a new centrifuge tube to test the effect of immunoprecipitation.**

(3) Add 1 mL 1×TBS, and re-suspend Anti-GST Magnetic Beads, place the tube on a Magnetic Separation Rack, let stand for 10 s, remove the supernatant, repeat 3-5 times, until OD280 of the supernatant is lesser than 0.05.

(4) Elution

a）Denatured elution: This method is suitable for SDS-PAGE and Western Blotting analysis of elution samples. Add 100 μL (5 times volume of Beads) 1×SDS-PAGE Loading Buffer (Dilute the SDS-page Loading Buffer (5×) by 5 times with 1×TBS) to the tube and mix well, incubate at 100°C for 5 min, then Centrifuge at 800 rpm for 1 min, and collect the supernatant to a new tube for SDS-PAGE and Western Blotting analysis.

b）Acid elution: This method can maintain their original biological activity, elution can be used for functional analysis. Add 100 μL (5 times volume of Beads) Elution Buffer to the tube and mix well, incubate at room temperature for 5-10 min (It is recommended to use vertical rotating mixer with Low-speed rotation), then centrifuge at 800 rpm for 2 min at 4°C, and collect the supernatant which is GST-Tag protein and its complex to a new tube, and immediately add 10 μL Neutralization Buffer to adjust the pH to 7.0-8.0. In order to improve the elution efficiency, elution can be repeated, and

combine the same samples. Place GST-Tag protein and its complex on ice to be used, or store at -20°C/-80°C for long-term. **It is recommended to add 100 μL 1×SDS-PAGE Loading Buffer to beads precipitation to test the effect of immunoprecipitation and elution.**

Note: a) For a few samples, due to differences in target proteins, the binding of GST-Tag and Anti-GST antibody is very strong, and the effect of Acid elution may be poor. Therefore, SDS-PAGE Loading Buffer denaturation elution method is recommended as a priority; b) Due to the difference of target protein, the elution efficiency of acid elution method also varies to some extent. If the requirement of elution efficiency is high, the pH value of acidic eluent can be adjusted appropriately between 2.5-3.1, and the pH value or quantity of corresponding neutralizing solution should be adjusted appropriately. For example, 100 μL Acid Elution Buffer (0.1 M Glycine-HCl, pH 2.8) and 15 μL Neutralizing Buffer (1 M Tris-HCl, pH 8.5).

Typical Data

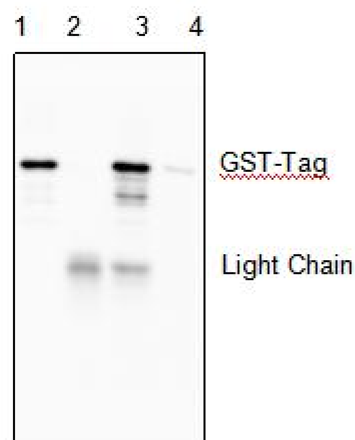

Fig. The immunoprecipitation effect of Anti-GST Magnetic IP Kit used for GST-Tag fusion protein.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.