

Anti-GST Magnetic Beads

cat : L-1014 Vol : 1ml

Product Description

The GST Tag Immunomagnetic Beads, conjugated with GST Antibody, are used for Immunoprecipitation / IP of GST-tagged proteins which expressed in vitro expression systems. For IP, the Immunomagnetic Beads are added to a sample containing GST-tagged proteins to form an Immunomagnetic Beads-protein complex. The complex is removed from the solution manually against a Magnetic Separator. The bound GST-tagged proteins are dissociated from the Immunomagnetic Beads using an Elution Buffer

Product Features

Composition	mouse IgG monoclonal Ab
Magnetization	Superparamagnetic
Particle size	200 nm
Concentration	10 mg/mL
Binding Capacity	≥ 0.6 mg GST-tagged fusion protein/mL of bead
Application	IP, CoIP, Pull down
Storage Condition	Store at 4°C for 2 years.

Protocol

1. Cell lysis

Cells may be lysed using any standard cell lysis protocol compatible with your starting material. We recommend the use of Cell Extraction Buffer or NP-40 Cell Lysis Buffer. Add protease inhibitor (such as PMSF at 1mM) if needed.

2. Preparation of Magnetic Beads

2.1 Resuspend the Magnetic Beads in the vial (tilt and rotate for 2 minutes or gently pipette for 10 times).

2.2 Transfer 20-100μL of Anti- GST Magnetic Beads into a 1.5 mL tube (Transfer amount may be adjusted as required).

2.3 Add 500 μL of binding/wash buffer to the beads and gently pipette to mix. Place the tube into a magnetic stand to collect the beads against the side of the tube

(Hereinafter referred to as magnetic separation). Remove and discard the supernatant. Repeat this step for 2 times.

3. Immunoprecipitation

3.1 Remove the tubes from the magnetic separator and add your sample containing GST-tagged protein to the pre-washed magnetic beads and incubate at room temperature for 2h or overnight at 4°C with mixing.

3.2 Collect the beads with a magnetic stand, remove the unbound sample and save for analysis.

3.3 Add 500μL of TBST to the tube and gently mix. Collect the beads and discard the supernatant. Repeat this wash twice.

3.4 Add 500μL of ultrapure water to the tube and gently mix. Collect the beads on a magnetic stand and discard the supernatant.

4. Elution

Chemical Elution Protocols

• Basic Elution

1. Add 100μL of 1x SDS-PAGE loading buffer to the tube.

2. Boil for 5 minutes on a dry bath.

3. Magnetically separate the beads and save the supernatant containing the target antigen.

• Acidic Elution

1. Add 100μL of 0.1M glycine, pH 3.0.

2. Gently vortex to mix and incubate the sample at room temperature on a rotator for 5-10 minutes.

3. Magnetically separate the beads and save the supernatant containing the target antigen.

4. To neutralize the low pH, add 15μL of Neutralization Buffer (1 M Tris pH 8.0) for each 100μL of eluate.

The final solution can be used as samples for denaturing SDS-PAGE. Or the elution can be adjusted to neutral pH with neutralization buffer immediately and used for further analysis.

Troubleshooting

Problem	Possible Cause	Solution
High background band	Non-specific binding of proteins to antibodies, insufficient washing on magnetic beads or EP tubes	The lysate was pretreated to remove non-specific proteins; before the last wash, the entire sample was transferred to a new EP tube and centrifuged.
	Not enough washing	Increase the time and number of washes
No protein band	No or minimal tagged protein was expressed	Verify protein expression; Prepare fresh lysate; Use the appropriate protease inhibitor.
	Insufficient incubation time	Increase incubation time
	Interfering substances in the sample	High concentrations of DTT, 2-mercaptoethanol or other reducing agents are present in the lysate.