



# **GST pull-down Kit**

**Catalog#JKR23011**

**Instruction Manual ( For Two Groups)**

Sufficient reagents for 6 GST pull-down assays per kit.

Store at -20 & 4°C

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## 1. Experimental Principle

The GST Pull-Down assay is based on glutathione-S-transferase (GST), which binds to glutathione (GSH) to immobilize GSH on magnetic beads, forming GSH-beads. Protein X is expressed as a fusion with GST, and the resulting GST-X protein can bind to GSH-beads. If protein Y, which interacts with protein X, is present in the system, a "bead-GSH-GST-X-Y" complex forms, allowing the interacting protein to be isolated and detected.

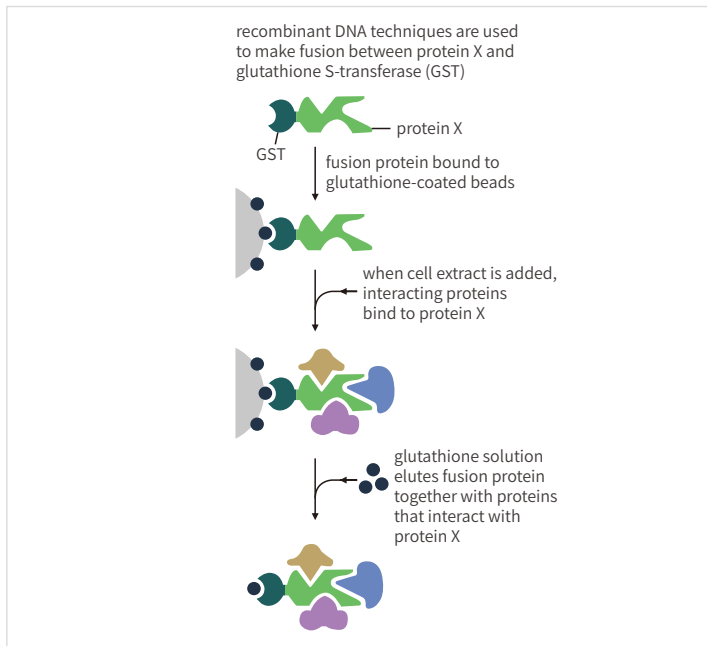


Figure 1: Schematic diagram of the GST pull-down experiment

## 2. Experimental Procedure

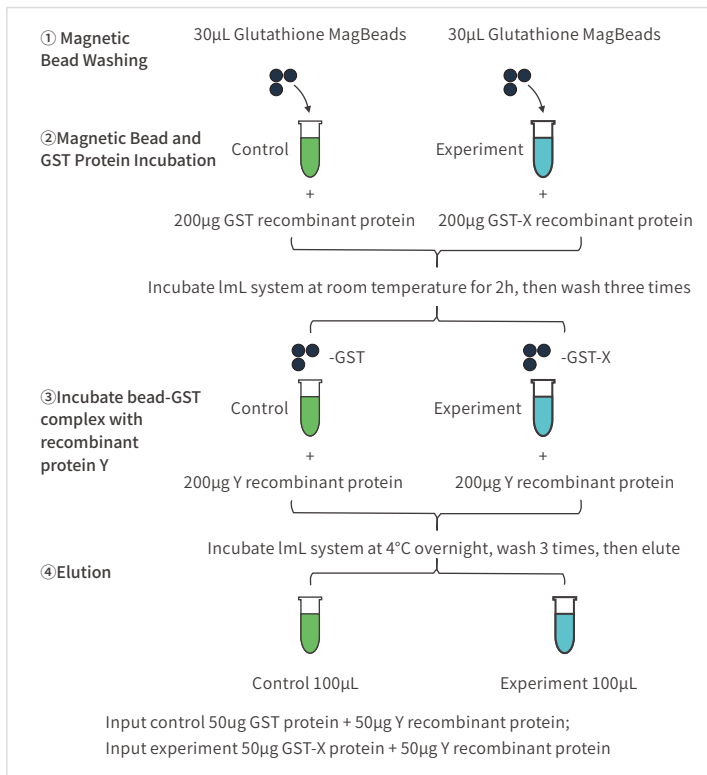


Figure 2: Validation of interaction between two known proteins

### 3. Kit Components

Component	Volume (6T)	Storage Temperature
Lysis buffer	9 mL	4°C
Glutathione MagBeads	200 $\mu$ L	4°C
Incubation buffer	12 mL	4°C
Washing buffer	30 mL	4°C
Elution buffer	800 $\mu$ L	4°C Protect from light
Protease inhibitor (100X)	35 $\mu$ L	-20°C

Special reminder 1: Reagents and consumables such as recombinant protein, PBS, 6X Loading buffer, magnetic rack, etc., need to be prepared by yourself.

Special reminder 2: 6T is for 6 single-group (1 control or 1 experimental) pull-down experiments; the subsequent steps include 1 control and 1 experimental group each, consuming 2T of reagent.

### 4. Operating Steps

#### 4.1 Protein extraction (optional; if verifying interaction between two recombinant proteins, skip protein extraction and start from step 4.2)

##### 4.1.1 Cell samples

- 1) Washing: Wash the sample (approximately  $2 \times 10^7$  cells) twice with 1 mL of pre-chilled PBS, and aspirate the PBS as thoroughly as possible after the last wash;
- 2) Lysis: Add 990  $\mu$ L Lysis buffer and 10  $\mu$ L Protease inhibitor (100X) according to cell count, fully lyse on ice for 20–30 min, inverting to mix once every 5 min;
- 3) Sonication: Sonicate for 5 min using an ultrasonic cell disruptor at 20% power, with 3 s on and 3 s off, in an ice bath;
- 4) Centrifugation: Centrifuge at 4°C, 12000 rpm for 10 min, and collect the supernatant.

##### 4.1.2 Tissue Samples

- 1) Grinding: Take fresh or cryopreserved tissue (approx. 0.3 g), place in a sterilized pre-cooled mortar, and grind to a powder using liquid nitrogen;
- 2) Lysis: Take 990  $\mu$ L Lysis buffer and 10  $\mu$ L Protease inhibitor (100X), mix to prepare lysis buffer, and aspirate. Add 800  $\mu$ L of lysis buffer to the mortar, continue grinding on ice for 5–10 min until the sample becomes a fine homogeneous slurry, transfer to a new 1.5 mL EP tube, then

- add the remaining 200  $\mu\text{L}$  of lysis buffer to the mortar to collect residual sample, and similarly transfer to this EP tube;
- 3) Allow the EP tube containing the sample slurry to fully lyse on ice for 30 min, inverting to mix once every 5 min;
  - 4) Ultrasonication: Sonicate using an ultrasonic cell disruptor for 8-10 min at 20% power, with 3 sec of sonication and 3 sec of pause, performed in an ice bath;
  - 5) Centrifugation: Centrifuge at 4°C, 12000 rpm for 10 min, collect the supernatant, then add Lysis buffer to the supernatant to bring the volume up to 1 mL, and mix well.

**Note:** The entire protein extraction process is performed on ice to reduce protein degradation caused by high temperatures; avoid bubble formation during sonication to minimize protein degradation. Store total protein at -20°C.

## 4.2 GST pull-down

### 4.2.1 Magnetic bead preparation

- 1) Take Glutathione MagBeads from the 4°C refrigerator, invert and mix several times to homogenize the beads and solution, then aliquot 30  $\mu\text{L}$  into two clean 1.5 mL Eppendorf tubes labeled as control and experimental;
- 2) Add 0.5 mL of pre-chilled washing buffer to resuspend the beads, place on a magnetic stand for 1 min to separate beads and solution, and carefully aspirate and discard the supernatant with a pipette.
- 3) Repeat step 2) twice, for a total of three washes.

### 4.2.2 Magnetic bead binding to GST-X protein

- 1) Add 200  $\mu\text{g}$  of GST-X recombinant protein to the experimental group; the control tube receives no protein or 200  $\mu\text{g}$  of GST recombinant protein. Adjust the volume to 1 mL with Incubation buffer, and incubate with gentle mixing at room temperature for 2 h.
- 2) Place both tubes on a magnetic stand for 1 min to separate the beads from the solution, and carefully aspirate and discard the supernatant with a pipette.
- 3) Add 0.5 mL of pre-chilled Washing buffer, place on a magnetic stand for 1 min to separate the beads from the solution, and carefully aspirate and discard the supernatant with a pipette.
- 4) Repeat step 3) twice, for a total of three washes.

### 4.2.3 Bait protein binding interaction protein Y (other recombinant proteins or total protein excluding GST tag)

- 1) Add 200  $\mu\text{g}$  of Y recombinant protein or 500-1000  $\mu\text{g}$  of total protein to two tubes respectively, supplement the volume to 1 mL with Incubation buffer, and incubate overnight (approximately 16 h) with gentle mixing at 4°C;

- 2) Place the 2 tubes on the magnetic stand for 1 min to separate the beads and solution, then carefully aspirate and discard the supernatant with a pipette;
- 3) Add 0.5 mL of pre-chilled Washing buffer, place on the magnetic stand for 1 min to separate the beads and solution, then carefully aspirate and discard the supernatant with a pipette;
- 4) Repeat step 3) twice, for a total of three washes.

#### 4.2.4 Elution

- 1) Add 100  $\mu$ L of Elution buffer to both tubes, incubate in a boiling water bath for 10 min, centrifuge at 12,000 rpm for 5 min, collect the supernatant, add 20  $\mu$ L of 6X Loading buffer, boil for 8-10 min, and label as the control group and experimental group;
- 2) Take two clean 1.5 mL EP tubes, labeled as Input control group and Input experimental group respectively. Add 50  $\mu$ g each of GST recombinant protein and Y recombinant protein to the Input control group, and add 50  $\mu$ g each of GST-X protein and Y recombinant protein to the Input experimental group (or add 100  $\mu$ L if using total protein). Add 1/5 volume of 6X Loading buffer, and boil in a water bath for 8-10 min.
- 3) Store the control group, experimental group, Input control, and Input experimental at  $-20^{\circ}\text{C}$  for later use.

#### 4.3 WB

Take 30  $\mu$ L each of the control, experimental, Input control, and Input experimental, and perform SDS-PAGE and Western blot detection.

#### 4.4 Silver Staining (Optional)

- 1) Take 30  $\mu$ L control, 30  $\mu$ L experimental, 2  $\mu$ L Input control, and 2  $\mu$ L Input experimental, and perform PAGE gel electrophoresis.
- 2) Fixation: Peel the electrophoresed gel from the glass plate, rinse it clean with water, place it in a clean 12 cm diameter glass dish, add deionized water to cover the gel, cover the dish, shake at room temperature on a destaining shaker for 5 min, discard the deionized water, add fixation solution to cover the gel, cover the dish, and shake at room temperature on a destaining shaker for 30 min.
- 3) Sensitization: Discard the fixative, add deionized water to cover the gel, cover with lid, shake at room temperature on a destaining shaker for 5 min, repeat washing with water once, for a total of 2 times. Add sensitization solution to cover the gel, cover with lid, shake at room temperature on a destaining shaker for 30 min.
- 4) Staining: Discard the sensitization solution, add deionized water to cover the gel, cover with lid, shake at room temperature on a destaining shaker for 2 min, repeat washing with water once, for a total of 2 times. Add staining solution to cover the gel, cover with lid, shake at

room temperature on a destaining shaker for 20 min.

- 5) Color development: Discard the staining solution, add deionized water to cover the gel, cover the lid, shake at room temperature on a destaining shaker for 1 min, repeat the water wash once, for a total of 2 times. Add developing solution to cover the gel, shake at room temperature on a destaining shaker for about 2 min until the solution turns turbid. Discard the liquid, add fresh developing solution and continue developing until the target bands are clear, then photograph.

## 4.5 Mass Spectrometry (Optional)

Take 30  $\mu\text{L}$  of control and experimental group protein samples for LC-MS detection.

## 5. Frequently Asked Questions

WB validation after pull-down revealed that although the target band is visible, the background is high:

Caused by various reasons:

- 1) Experimental instruments or reagents are contaminated; use clean instruments and reagents.
- 2) High background due to non-specific adsorption on the membrane; wear gloves during experimental operation, use tweezers to handle, and avoid touching the transfer surface of the membrane.
- 3) If large protein complexes in the sample are not fully dissolved, briefly sonicate after preparation (3 times, 5 sec each), then centrifuge and use the supernatant for subsequent experiments.
- 4) Inadequate washing; increase the number of washes and raise the concentration of NaCl and detergent in the wash solution.
- 5) High background from using excessive cells or tissue for lysis; reduce sample amount, with 100-500 $\mu\text{g}$  of cell lysate recommended.
- 6) Protein degradation may also cause high background; use freshly prepared samples whenever possible.



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