(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSTICS!)

Elabscience® GST pull-down Kit (Agarose)

Product code：EA-IP-K008

Product specifications：25 T

Please read the instructions carefully before use. If you have any questions, please contact us at:

Tel: 240-252-7368(USA)

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Please refer to the outer package label of the kit for the specific shelf life and use the kit within its shelf life.

Please provide the product lot number (see kit label) when contacting so that we can serve you more efficiently.

Background Information

The GST Pull-Down experiment is based on the reversible specific binding between glutathione-S-transferase (GST) and glutathione (GSH). The corresponding target protein is isolated and purified by GST fused bait protein. The bait protein fuses with GST, and the GST-bait fusion protein binds to GSH-agarose gel complex. If the target protein interacts with the bait protein in the environment, the "agarose gel-GSH-GST-bait protein-target protein" complex will be formed, and then the target protein interacting with the bait protein can be separated and detected.

This product is made of high-quality reduced glutathione (GSH) and agarose gel by covalent binding. It has the characteristics of high binding capacity of protein, fast and convenient operation, strong specificity and specificity, and can specifically combine with proteins containing GST tags in cells or microbial lysates.

Performance Index

1. Scope of application

It is derived from the pull down experiment of cells or microbial lysates containing GST labeled fusion protein.

2. Binding properties

Using 4% agarose gel as the matrix, through the spacer arm of 12 atoms, it is made by chemical covalently combining reduced glutathione.

3. Gel properties

Agarose gel particles, average particle size 50 μm.

4. Composition

1 mL of covalently bound GSH gel was stored in 1 mL of PBS containing preservative and 50% glycerol.

Product Components

Cat. No.	Component	Code	Specification	Storage
E-IR-IP002	Centrifugal Column	C	0.5mL × 27	Room temperature, 12 months
EA-IP-008	GSH-agarose	G1	2mL	4°C, 12 months
E-IR-IP004	Lysis buffer	L1	30mL	4°C, 12 months
E-AB-48005	GST monoclonal antibody	A1	50μL	-20°C, 12 months
E-IR-IP008	Glutathione	E3	1g	4°C, 12 months
E-BC-R187	PBS Buffer, pH7.4 (10×)	P10	50mL	4°C, 12 months
E-IR-R310	PBST Buffer,pH7.4 (10×)	P10T	50mL	4°C, 12 months
Manual	one copy			

Matters Needing Attention

1. Transportation and storage:

This kit is transported under refrigerated conditions.

After receiving the goods, if it is not used temporarily, please take out the centrifugal column **C** and store it at room temperature; take out GST monoclonal antibody **A1** and store at - 20 ℃; The rest of the kit components can be stored at 4 °C。

2. Suggestions on reagent use

P10 (PBS Buffer, pH 7.4 (10×)) and **P10T** (PBST Buffer, pH 7.4 (10×)) should be diluted into 1x working solution with deionized water before use.

3. Affinity gel suspension

The kit provides affinity gel in the form of a gel suspension with a 50% affinity gel content. Gently re-suspended the gel suspension before use and use as required.

For example, 2mL of gel suspension contains 1mL of affinity gel.。

4. Suggestions for using gel

Do not freeze or dry the gel, do not use sonication for the gel, and acid treatment time of the gel should not exceed 10min.

Self-Prepared Reagent

1. 1× PBS

Dilute **P10** (PBS Buffer, pH 7.4 (10×)) with deionized water in a ratio of 9:1 for use. For example, add 1mL of **P10** into 9mL of deionized water and mix it to 1× PBS. Prepare when needed.

2. 1× PBST

Dilute **P10T** (PBST Buffer, pH 7.4 (10×)) with deionized water in a ratio of 9:1 for use. For example, add 9mL of deionized water to 1mL **P10T** and mix it to 1× PBST. Prepare when needed.

3. Eluent

Weigh 0.6g Tris base, 0.88g NaCL, 0.31g glutathione **E3**, and dissolve them in 10ml deionized water to make 10x eluent.

Dilute 10x eluent into 1x working solution with deionized water at a ratio of 9:1 for use. Prepare when needed.

Method of Application

Note: All steps are performed on ice as much as possible to avoid degradation of target proteins. The amount of reagent used below is calculated in a gel volume of 40μL, binding 100-150ug GST- fusion protein from the cell supernatant. You can also adjust the reagent dosage proportionally according to the specific gel dosage.

40μL GSH-agarose can bind up to 200μg of GST-fused proteins. However, due to differences in conformation, GST tag fusion proteins may exhibit lower binding capacity. In addition, large molecular weight fusion proteins may prevent the binding of GST tags to GSH on the gel due to steric hindrance.

1. Preparation of bait protein

1) Secretory GST tag fusion protein

Collect the supernatant and detect the concentration of bait protein. If the bait protein concentration is high, it is recommended to dilute it with 1xPBS until the final protein concentration is 500 μg/mL for subsequent experiments.

2) GST tag fusion protein expression in mammalian cells

a) Collecting cells

Blow the suspended cells and semi-adherent cells off the cell culture flask and transfer them into a centrifuge tube, centrifuge at 1000rpm for 5min, and discard the supernatant.

Gently scrape the adherent cells off the bottle wall with a cell scraper, transfer them into a centrifuge tube together with the culture medium, centrifuge at 1000rpm for 5min, and discard the supernatant.

b) Re-suspend the cells with 1x PBS pre-cooled to 4 °C, centrifuge at 1000rpm for 3min, and discard the supernatant. Repeat.

c) Add the corresponding volume of lysis buffer according to the number of cells, and place it on the ice for 10-20min after repeated blowing.

Note: Generally, 1mL of lysis buffer can process about 0.5–1 x10^7 cells. To avoid degradation of that target protein, you may add protease inhibitor.

d) Treat cell lysate with ultrasonic crusher until cell lysate is clear and no longer viscous. After 30 min on ice, centrifuge at 12000 rpm for 10 min at 4 °C. Take the supernatant for subsequent experiments.

3) GST tag fusion protein expression in *Escherichia coli*

Note: The purification of GST fused proteins should always be maintained under non-denatured conditions. If the fusion protein is expressed as an inclusion body, after the inclusion body is dissolved with 8M urea or 6M guanidine hydrochloride, the urea and guanidine hydrochloride should be removed by dialysis and the protein can be regenerated before the product can be purified.

a) Collect the bacterial cells by centrifuging at 4 °C and 4000 rpm for 15min, discard the supernatant. Add an appropriate amount of PBST to re-suspend the cells, add PMSF as a protease inhibitor at a final concentration of 1mM. Break the cells with sonication under ice bath conditions.

b) Perform high speed centrifugation at 4 °C, and 8000rpm for 15min, and take the supernatant into a new EP tube for subsequent experiments.

4) Purified GST fusion protein

Detect the concentration of bait protein. If the bait protein concentration is high, it is recommended to dilute it with 1xPBS until the final protein concentration is 500 μg/mL for subsequent experiments.

Note: If the bait protein contains GSH, it needs to be removed by dialysis or ultrafiltration before the subsequent experimental steps.

2. Target protein identification by Pull-down method

1) Column loading and bait protein fixation

a) Gently re-suspended GSH agarose gel, mix evenly, and aspirate 80 μL gel suspension (about 40 μL gel) into the centrifuge tube with the pipette (cut off the tip head). Wash the affinity gel with 600 μL 1xPBS, centrifuge at 1000 rpm for 30 seconds, discard the supernatant, and repeat this step twice.

b) Add the bait protein obtained in step **1** into the pre washed gel, mix it gently and incubate it on a shaker at 4°C for 3h. A bait protein sample should be retained here as input controls for future testing.

c) Transfer the complex in the centrifuge tube to the centrifugal column, centrifuge at 1000rpm for 5min, transfer the centrifuge liquid to a new tube, and mark it as bait protein flow through solution for subsequent detection.

Note: For cell or bacterial culture, add at least 500 μL GST labeled fusion protein lysate. For the purified GST tag fusion protein, use enough volume to ensure that about 100-150 μg of bait protein is added.

d) Add 500 μL 1xPBS to the centrifugal column, invert for several times, mix gently, wash the gel, centrifuge at 1000 rpm for 30 seconds, and discard the supernatant. Repeat four times. Get bait protein gel complex.

2) Target protein binding to bait protein

a) Add 800 μL of the prepared target protein lysate to the bait protein gel complex in spin column from step (2-1-d). Shake gently at 4°C for at least 3 hours or incubate overnight. A target protein sample

should be retained here as target protein input for subsequent detection.

Note: In order to ensure the sufficient binding of bait protein and capture protein, longer incubation time may be required, which can be adjusted according to the experimental situation.

b) After incubation, centrifuge this complex at 1000 rpm for 30 seconds, transfer the centrifuged liquid to a new centrifuge tube, and mark it as target protein flow through solution for subsequent detection.

c) Add 500 μL 1xPBST to the spin column, invert it for several times and mix it gently, wash the gel, centrifuge it at 1000 rpm for 30 seconds, and discard the centrifugal solution. Repeat four times. Get bait capture protein gel complex。

3) Elution bait-target protein complex

This manual provides the following two protein elution schemes. Please select elution method according to the needs of later detection.

a) Denaturation elution method: The samples eluted by this method are suitable for SDS-PAGE detection.

Transfer an appropriate amount of gel to a 1.5ml centrifuge tube, centrifuge, discard the supernatant, add 2x sample loading buffer to the gel at 1:1 ratio, mix evenly, and boil for 5 minutes. Centrifuge the gel, collect supernatant, and perform SDS-PAGE or Western blot analysis.

b) Non denaturing elution method: The protein eluted by this method can be used for later functional analysis.

Add 250-500 μL eluent to the centrifuge column, incubate at room temperature for 10 min; Replace a new collecting tube, centrifuge at 1000 rpm for 30 seconds, collect the flowing liquid into the new

collecting tube, perform dialysis, the sample after dialysis can be used for later functional analysis.

c) This kit provides GST mouse monoclonal antibody. You can prepare working solution at a ratio of 1:10000 for WB detection of bait protein.

Declaration

1. This product is limited to the scientific use of a professional.
2. Please pay attention to safety matters, and follow the laboratory reagent operation specification.
3. The lysis buffer provided by the kit is a formula repeatedly optimized for a long time and verified by a large number of experiments. When treating cells, it is recommended to use the lysis buffer matching with this kit, and the lysis buffer provided by other manufacturers may affect protein co-precipitation or the results of subsequent IP experiments.
4. The conditions recommended in this specification are general. The user can optimize the experimental conditions and select the most appropriate experimental protocol according to the properties of different target proteins.