Gαi活性检测试剂盒丨免疫检测试剂盒

产品名称	Gαi活性检测试剂盒丨免疫检测试剂盒
公司名称	武汉费斯德生物科技有限公司
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产品详情

Gαi Pull-Down Activation Assay Kit

Cat. # 83001

IntroductionA. Background

A structurally diverse repertoire of ligands, from photons to large peptides, activates G protein-coupled receptors (GPCRs) to elicit their physiological functions. Ligand-bound GPCRs, in turn, function as guanine nucleotide exchange factors catalyzing the exchange of GDP bound on the Gα subunit with GTP in the presence of Gβγ, causing the dissociation of the Gα subunit from the Gβγ dimer to form two functional units (Gα and Gβγ). Both Gα and Gβγ subunits signal to various cellular signaling pathways. Based on the sequence and functional homologies, G proteins are grouped into four families: Gs, Gi, Gq, and G12.

Gαi family is the largest family of G proteins. They relay signals from many GPCRs to regulate various biological functions. There were no direct methods to measure the activation of Gαi Proteins by receptors (until this assay kit). Most reports used one of the downstream pathways, i.e. the inhibition of adenylyl cyclases, as a readout. Alternatively, sensitivity to pertussis toxin (PTX) was used as an indicator of possible Gαi proteins involved in a signaling pathway.

B. Assay Principle

The Gαi Activation Assay Kit uses configuration-specific anti-Gαi-GTP Mouse monoclonal antibody to measure Gαi-GTP levels in cell extracts or in vitro GTPγS loading Gαi activation assays. Anti-Gαi-GTP mouse monoclonal antibody is first incubated with cell lysates containing Gαi-GTP. Next, the GTP-bound Gαi is pulled down by protein A/G agarose. Finally, the precipitated Gαi-GTP is detected through immunoblot analysis using anti-Gαi mouse monoclonal antibody.

C. Kit Components

1. Anti-G α i-GTP Mouse Monoclonal Antibody (Cat. # 26901): One vial – 35 L (1 mg/ml) in PBS, pH 7.4, containing 50% glycerol. This antibody specifically recognizes G α i-GTP from all vertebrates.

2. Protein A/G Agarose (Cat. # 30301): One vial – 600 L of 50% slurry.

3. 5X Assay/Lysis Buffer (Cat. # 30302): One bottle – 30 mL of 250 mM Tris-HCl, pH 8, 750mM NaCl, 50 mM MgCl2, 5 mM EDTA, 5% Triton X-100.

4. Anti-G α i Mouse monoclonal Antibody (Cat. # 26003): One vial – 50 L (1mg/mL) in PBS, pH 7.4, contained 50% glycerol.

5. 100X GTP γ S (Cat. # 30303): One vial – 50 l at 10 mM, use 5 L of GTP γ S for GTP-labeling of 0.5 mL of cell lysate.

6. 100X GDP (Cat. # 30304): One vial – 50 l at 100 mM, use 5 L of GDP for GDP-labeling of 0.5 mL of cell lysate.

7. HRP-Goat Anti-Rabbit IgG (Cat. #29002): 50 L (0.4 mg/mL) in PBS, pH 7.4, contained 50% glycerol.

D. Materials Needed but Not Supplied

1. Stimulated and non-stimulated cell lysates

2. Protease inhibitors

3. 4 ° C tube rocker or shaker

4. 0.5 M EDTA at pH 8.0

5. 1.0 M MgCl2

6. 2X reducing SDS-PAGE sample buffer

7. Electrophoresis and immunoblotting systems

8. Immunoblotting wash buffer such as TBST (10 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 0.05% Tween-20)

9. Immunoblotting blocking buffer (TBST containing 5% Non-fat Dry Milk or 3% BSA)

10. ECL Detection Reagents

E. Example Results

The following figure demonstrates example results seen with the G α i Activation Assay Kit. For reference only.

G α i Activation Assay.

A. CHO cells were transfected with wild-type G α i1 (lanes 1 and 2) or constitutively active G α i1-Q204L (lane 3). Cell lysates were treated with GDP (lane 1) or GTP γ S (lane 3). Lysates were then incubated with an anti-G α i-GTP

monoclonal antibody (Cat. # 26901) (top panel). The precipitated G α i-GTP was immunoblotted with an anti-G α i monoclonal antibody (Cat. # 26003). The bottom panel shows the Western blot with anti-G α i monoclonal antibody (Cat. # 26003) of the cell lysates. B. HEK293 cells stably expressing human m2 mAChR were treated with (lane 2) or without (lane 1) carbachol. Cell lysates were then incubated with an anti-active G α i monoclonal antibody (Cat. No. 26901) (top panel). The precipitated G α i-GTP was immunoblotted with an anti-G α i rabbit polyclonal antibody (Cat. # 21006). The bottom panel shows the Western blot with anti-tubulin of the cell lysates.

Assay ProcedureA. Reagent Preparation

1X Assay/Lysis Buffer: Mix the 5X Stock (Cat. # 30301) briefly and dilute to 1X in deionized water. Just prior to usage, add protease inhibitors such as 1 mM PMSF, 10 g/mL leupeptin, or 10 g/mL aprotinin.

B. Sample PreparationAdherent Cells

1. Culture cells (one 10-cm plate, ~107 cells) to approximately 80-90% confluence. Stimulate the cells with activator or inhibitor as desired.

2. Aspirate the culture media and wash twice with ice-cold PBS.

3. Completely remove the final PBS wash and add ice-cold 1X Assay/Lysis Buffer (See Reagent Preparation) to the cells (0.5-1 mL per 10 cm tisue culture plate).

4. Place the culture plates on ice for 10-20 minutes.

5. Detach the cells from the plates by scraping with a cell scraper.

6. Transfer the lysates to appropriate size tubes and place on ice.

7. If nuclear lysis occurs, the cell lysates may become viscous and difficult to pipette. If this occurs, lysates can be passed through a 27-gauge syringe needle 3-4 times to shear the genomic DNA.

8. Clear the lysates by centrifuging at 12,000 x g and 4 ° C for 10 minutes.

9. Collect the supernatant and store the sample (~1-2 mg of total protein) on ice for immediate use, or snap freeze and store at -70 ° C for future use.

Adherent Cells

1. Culture cells and stimulate with activator or inhibitor as desired.

2. Perform a cell count and then pellet the cells through centrifugation.

3. Aspirate the culture media and wash twice with ice-cold PBS.

4. Completely remove the final PBS wash and add ice-cold 1X Assay/Lysis Buffer (See Reagent Preparation) to the cell pellet (0.5-1 mL per 107 cells).

5. Lyse the cells by repeated pipetting.

6. Transfer the lysates to appropriate size tubes and place them on ice.

9. Collect the supernatant and store sample on ice for immediate use, or snap freeze and store at -70 ° C for future use.

C. In vitro GTP γ S/GDP Protein for Positive and Negative controls

Note: In vivo stimulation of cells will activate approximately 10% of the available G α i, whereas in vitro GTP γ S protein loading will activate nearly 90% of G α i.

1. Aliquot 0.5 mL of cell extract (or 1 g of purified G α i protein) into two microcentrifuge tubes.

2. To each tube, add 20 L of 0.5 M EDTA (final concentration of 20 mM).

3. Positive control: add 5 L of 100 X GTP γ S (Cat. # 30302) to the 1st tube

4. Negative control: add 5 L of 100 X GDP (Cat. # 30304) to the 2nd tube.

5. Incubate both tubes at 30 ° C for 30 minutes with agitation.

6. Stop loading by placing the tubes on ice and adding 32.5 L of 1 M MgCl2 (final concentration of 60 mM).

D. Affinity Precipitation of Activated G Protein

1. Aliquot 0.5-1 mL of cell lysates (about 1 mg of total cellular protein) to a microcentrifuge tube.

2. Adjust the volume to 1 mL with 1X Assay/Lysis Buffer (See Reagent Preparation).

3. Add 1 L anti-G α i-GTP antibody (Cat. # 26901).

4. Prepare the protein A/G Agarose bead slurry (Cat. # 30301) by resuspending through vertexing or titrating.

5. Quickly add 20 L of resuspended bead slurry to above tube.

6. Incubate the tube at 4 ° C for 1 hour with gentle agitation.

7. Pellet the beads through centrifugation at 5,000 x g for 1 min.

8. Aspirate and discard the supernatant (making sure not to disturb or remove the bead pellet.

9. Wash the beads 3 times with 0.5 mL of 1X Assay/Lysis Buffer, centrifuging and aspirating each time.

10. After the third wash, pellet the beads through centrifugation and carefully remove all the supernatant.

11. Resuspend the bead pellet in 20 L of 2X reducing SDS- PAGE sample buffer.

12. Boil the sample for 5 minutes.

13. Centrifuge it at 5,000 x g for 10 seconds.

E. Western Blot Analysis

1. Load 15 L/well of pull-down supernatant to a polyacrylamide gel (17%). It is recommended to include a pre-stained MW standard (as an indicator of a successful transfer in step 3 below).

2. Perform SDS-PAGE following the manufacturer ' s instructions.

3. Transfer the gel proteins to a PVDF or nitrocellulose membrane following the manufacturer ' s instructions.

Note: Steps 4-11 are at room temperature with agitation

4. Following electroblotting, immerse the PVDF membrane in Methanol for 15 seconds, and then allow it to dry at room temperature for 5 minutes.

Note: If Nitrocellulose is used instead of PVDF, step 4 Should be skipped.

5. Block the membrane with 5% non-fat dry milk or 3% BSA in TBST for 1 he at room temperature with constant agitation.

6. Wash the blotted membrane three times with TBST, 5 minutes each time.

7. Incubate the membrane with anti-G α i Mouse Monoclonal Antibody (Cat. # 26003), which has been freshly diluted 1: 50~500 (depending on the amount of G α i proteins in your sample) in 5% non-fat dry milk or 3% BSA in TBST, for 1-2 her at room temperature with constant agitation or at 4 ° C overnight.

8. Wash the blotted membrane three times with TBST, 5 minutes each time.

9. Incubate the membrane with a secondary antibody (Cat. # 29002), which has been freshly diluted 1: 1000 in 5% non-fat dry milk or 3% BSA in TBST, for 1 he at room temperature with constant agitation.

10. Wash the blotted membrane three times with TBST, 5 minutes each time.

11. Use the detection method of your choice such as ECL.