GA$_i$ PULL-DOWN ACTIVATION ASSAY KIT

Gα$_i$ Pull-Down Activation Assay Kit

Cat. # 83001

Introduction

A. 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.
Gα$_i$ 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 Gα$_i$ 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 Gα$_i$ proteins involved in a signaling pathway.

B. Assay Principle

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

C. Kit Components

1. Anti-Gα$_i$-GTP Mouse Monoclonal Antibody (Cat. # 26901): 30 µL (1 mg/ml) in PBS, pH 7.4, containing 50% glycerol. This antibody specifically recognizes Gα$_i$-GTP from

all vertebrates.
2. Protein A/G Agarose (Cat. # 30301): 600 µL of 50% slurry.
3. 5X Assay/Lysis Buffer (Cat. # 30302): 30 mL of 250 mM Tris-HCl, pH 8, 750 mM NaCl, 50 mM MgCl2, 5 mM EDTA, 5% Triton X-100.
4. Anti-$G\alpha_i$ Mouse monoclonal Antibody (Cat. # 26003): 50 µL (1mg/mL) in PBS, pH 7.4, contained 50% glycerol.
5. 100X GTPγS (Cat. # 30303): 50 µl at 10 mM, use 5 µL of GTPγS for GTP-labeling of 0.5 mL of cell lysate.
6. 100X GDP (Cat. # 30304): 50 µl at 100 mM, use 5 µL of GDP for GDP-labeling of 0.5 mL of cell lysate.
7. HRP-Goat Anti-Rabbit IgG (Cat. # 29002): 50 µL (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 $MgCl_2$
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 $G\alpha_i$ Activation Assay Kit. For reference only.