GA_s PULL-DOWN ACTIVATION ASSAY KIT

$G\alpha_s$ Pull-Down Activation Assay Kit

Cat. # 80801

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.
$G\alpha_s$ family relays signals from many GPCRs to regulate various biological functions such as the stimulation of adenylyl cyclases. There were no direct methods to measure the activation of $G\alpha_s$ proteins by receptors (until this assay kit). Most reports used one downstream pathway, the increase of cAMP, as a readout.
$G\alpha_s$ Activation Assay Kit is based on the monoclonal antibody specifically recognizing the active GTP-bound $G\alpha_s$ proteins. This monoclonal antibody has much lower affinity towards the inactive $G\alpha_s$ proteins. Therefore, after activation by receptor signals, active GTP-bound $G\alpha_s$ proteins could be immunoprecipitated by this monoclonal antibody and further quantified by western blot with another anti-$G\alpha_s$ antibody.

B. Assay Principle

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

C. Kit Components

1. Anti-$G\alpha_s$-GTP Mouse Monoclonal Antibody (Cat. # 26906): 30 µL (1 mg/ml) in PBS, pH 7.4, containing 50% glycerol. This antibody specifically recognizes $G\alpha_s$-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-$G\alpha_s$ Mouse monoclonal Antibody (Cat. # 26006): 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 µg/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 ºC 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 $G\alpha_s$ Activation Assay Kit. For reference only.