

GA_s PULL-DOWN ACTIVATION ASSAY KIT**Gα_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. Gα_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 Gα_s proteins by receptors (until this assay kit). Most reports used one downstream pathway, the increase of cAMP, as a readout. Gα_s Activation Assay Kit is based on the monoclonal antibody specifically recognizing the active GTP-bound Gα_s proteins. This monoclonal antibody has much lower affinity towards the inactive Gα_s proteins. Therefore, after activation by receptor signals, active GTP-bound Gα_s proteins could be immunoprecipitated by this monoclonal antibody and further quantified by western blot with another anti-Gα_s antibody.

B. Assay Principle

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

C. Kit Components

1. Anti- $G\alpha_s$ -GTP Mouse Monoclonal Antibody (Cat. # 26906): 30 μ L (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 μ L of 50% slurry.
3. 5X Assay/Lysis Buffer (Cat. # 30302): 30 mL of 250 mM Tris-HCl, pH 8, 750 mM NaCl, 50 mM $MgCl_2$, 5 mM EDTA, 5% Triton X-100.
4. Anti- $G\alpha_s$ Mouse monoclonal Antibody (Cat. # 26006): 50 μ L (1mg/mL) in PBS, pH 7.4, contained 50% glycerol.
5. 100X GTP γ S (Cat. # 30303): 50 μ L at 10 mM, use 5 μ L of GTP γ S for GTP-labeling of 0.5 mL of cell lysate.
6. 100X GDP (Cat. # 30304): 50 μ L at 100 mM, use 5 μ L of GDP for GDP-labeling of 0.5 mL of cell lysate.
7. HRP-Goat Anti-Rabbit IgG (Cat. # 29002): 50 μ L (0.4 μ g/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 $G\alpha_s$ Activation Assay Kit. For reference only.