

GA_z PULL-DOWN ACTIVATION ASSAY KIT**Gα_z Pull-Down Activation Assay Kit****Cat. # 81001****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 (including Gα_z) 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_z 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.

Gα_z Activation Assay Kit is based on the monoclonal antibody specifically recognizing the active GTP-bound Gα_z proteins. This monoclonal antibody has much lower affinity towards the inactive Gα_z proteins. Therefore, after activation by receptor signals, active GTP-bound Gα_z proteins could be immunoprecipitated by this monoclonal antibody and further quantified by western blot with another anti-Gα_z antibody.

B. Assay Principle

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

C. Kit Components

1. Anti-G α_z -GTP Mouse Monoclonal Antibody (Cat. # 26908): 30 μ L (1 mg/ml) in PBS, pH 7.4, containing 50% glycerol. This antibody specifically recognizes G α_z -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 MgCl₂, 5 mM EDTA, 5% Triton X-100.
4. Anti-G α_z Mouse monoclonal Antibody (Cat. # 26011): 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 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_z Activation Assay Kit. For reference only.