

Product Description

Pioneering GTPase and Oncogene Product Development since 2010

GA, 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 G α subunit with GTP in the presence of G $\beta\gamma$, causing the dissociation of the G α subunit from the G $\beta\gamma$ dimer to form two functional units (G α and G $\beta\gamma$). Both G α and G $\beta\gamma$ 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 α 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 G α 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 G α proteins involved in a signaling pathway.

B. Assay Principle

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



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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-Gα_i Mouse monoclonal Antibody (Cat. # 26003): 50 μL (Img/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 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 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 $G\alpha_i$ Activation Assay Kit. For reference only.