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**Product Manual**

## Configuration-specific Monoclonal Antibody Based

# $G\alpha_{13}$ Activation Assay Kit

**Catalog Number：80401**

**20 assays**

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**FOR RESEARCH USE ONLY.**
**NOT FOR USE IN DIAGNOSTIC PROCEDURES**

## Product Description

A structurally diverse repertoire of ligands, from photons to large peptides, activates 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: $G_s$, $G_i$, $G_q$, and $G_{12}$ . As increasing numbers of effectors and interacting proteins for these G proteins have been identified, the physiological processes in which G proteins participate are multiplying.

Among the four subfamilies of G proteins, the function of $G_{12/13}$ subfamily is less well understood. In this family, there are two members, $G_{12}$ and $G_{13}$, that are expressed ubiquitously. $G\alpha_{12}$ knockout mice appeared normal. $G\alpha_{13}$ knockout mice displayed embryonic lethality (~E9.5). The $G\alpha_{13}^{-/-}$ mouse embryos had defective vascular systems. $G_{13}$ is also essential for receptor tyrosine kinase-induced migration of fibroblast and endothelial cells.

NewEast Biosciences $G\alpha_{13}$ Activation Assay Kit provides a simple and fast tool to monitor the activation of $G\alpha_{13}$. Each kit provides sufficient quantities to perform 20 assays.

## Assay Principle

NewEast Biosciences $G\alpha_{13}$ Activation Assay Kit bases on the configuration-specific anti-$G\alpha_{13}$-GTP monoclonal antibody to measure the active $G\alpha_{13}$-GTP levels, either from cell extracts or from in vitro GTPγS loading $G\alpha_{13}$ activation assays. Briefly, anti-active $G\alpha_{13}$ mouse monoclonal antibody will be incubated with cell lysates containing $G\alpha_{13}$-GTP. The bound active $G\alpha_{13}$ will then be pulled down by protein A/G agarose. The precipitated active $G\alpha_{13}$ will be detected by immunoblot analysis using anti-$G\alpha_{13}$ rabbit polyclonal antibody.

## Kit Components

1. Anti-active $G\alpha_{13}$, Mouse Monoclonal Antibody (Catalog No. 26902): One vial – 22 µL (1 mg/ml) in PBS, pH 7.4, containing 50% glycerol and 0.05% sodium azide. This antibody specifically recognizes $G\alpha_{13}$-GTP from all vertebrates.

2. Protein A/G Agarose (Catalog No. 30301): One vial – 400 µL of 50% slurry.

3. 5X Assay/Lysis Buffer (Catalog No. 30303): One bottle – 30 mL of 250 mM Tris-HCl, pH 8, 750 mM NaCl, 5 mM EDTA, 5% Triton X-100.

4. Anti- $G\alpha_{13}$, Rabbit Polyclonal Antibody (Catalog No. 21005): One vial – 22 µL (1 mg/ml) in PBS, pH 7.4, contained 50% glycerol.

## Storage

Store all kit components at 4°C until their expiration dates.

## Materials Needed but Not Supplied

1. Stimulated and non-stimulated cell lysates
2. Protease inhibitors
3. 4 °C tube rocker or shaker
4. 1 M EDTA, pH8.0
5. 1 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. PVDF or nitrocellulose membrane
11. Secondary Antibody
12. ECL Detection Reagents

## Reagent Preparation

- 1X Assay/Lysis Buffer: Mix the 5X Stock briefly and dilute to 1X in deionized water. Just prior to usage, add protease inhibitors such as 1 mM PMSF, 10 µg/mL leupeptin, and 10 µg/mL aprotinin.

## Sample Preparation

### Adherent Cells

1. Culture cells to approximately 80-90% confluence. Stimulate cells with activator or inhibitor as desired.

2. Aspirate the culture media and wash twice with ice-cold PBS.
3. Completely remove the final PBS wash and add ice-cold 1X Assay/Lysis Buffer to the cells (0.5 - 1 mL per 100 mm tissue culture plate).
4. Place the culture plates on ice for 10-20 minutes.
5. Detach the cells from the plates by scraping with a cell scraper.
6. Transfer the lysates to appropriate size tubes and place on ice.
7. If nuclear lysis occurs, the cell lysates may become very viscous and difficult to pipette. If this occurs, lysates can be passed through a 27½-gauge syringe needle 3-4 times to shear the genomic DNA.
8. Clear the lysates by centrifugation for 10 minutes (12,000 x g at 4 °C).
9. Collect the supernatant and store samples on ice for immediate use, or snap freeze and store at -70 °C for future use.

**Suspension Cells**

1. Culture cells and stimulate with activator or inhibitor as desired.
2. Perform a cell count, and then pellet the cells by centrifugation.
3. Aspirate the culture media and wash twice with ice-cold PBS.
4. Completely remove the final PBS wash and add ice-cold 1X Assay/Lysis Buffer to the cell pellet (0.5 – 1 mL per 1 x $10^7$cells).
5. Lyse the cells by repeated pipetting.
6. Transfer the lysates to appropriate size tubes and place on ice.
7. If nuclear lysis occurs, the cell lysates may become very viscous and difficult to pipette. If this occurs, lysates can be passed through a 27½-gauge syringe needle 3-4 times to shear the genomic DNA.
8. Clear the lysates by centrifugation for 10 minutes (12,000 x g at 4 °C).
9. Collect the supernatant and store samples on ice for immediate use, or snap freeze and store at -70 °C for future use.

# Assay Procedure

### I. Active $G\alpha_{13}$ Pull-Down Assay

1. Aliquot 0.5 – 1 mL of cell lysate to a microcentrifuge tube.
2. Adjust the volume of each sample to 1 mL with 1X Assay/Lysis Buffer.
3. Add 1 µl anti-active $G\alpha_{13}$ monoclonal antibody to the tube.

4. Thoroughly resuspend the protein A/G Agarose bead slurry by vortexing or titurating.
5. Quickly add 20 µL of resuspended bead slurry to each tube.
6. Incubate the tubes at 4 °C for 1 hour with gentle agitation.
7. Pellet the beads by centrifugation for 1 min at 5,000 x g.
8. Aspirate and discard the supernatant, making sure not to disturb/remove the bead pellet.
9. Wash the bead 3 times with 0.5 mL of 1X Assay/Lysis Buffer, centrifuging and aspirating each time.
10. After the last wash, pellet the beads and carefully remove all the supernatant.
11. Resuspend the bead pellet in 20 µL of 2X reducing SDS-PAGE sample buffer.
12. Boil each sample for 5 minutes.
13. Centrifuge each sample for 10 seconds at 5,000 x g.

## II. Electrophoresis and Transfer

1. Load 15 µL/well of pull-down supernatant to a polyacrylamide gel (8%). Also, it's recommended to include a pre-stained MW standard (as an indicator of a successful transfer in step 3).
2. Perform SDS-PAGE following the manufacturer's instructions.
3. Transfer the gel proteins to a PVDF or nitrocellulose membrane following the manufacturer's instructions.

## III. Immunoblotting and Detection (all steps are at room temperature, with agitation)

1. Following the electroblotting step, immerse the PVDF membrane in 100% Methanol for 15 seconds, and then allow it to dry at room temperature for 5 minutes.

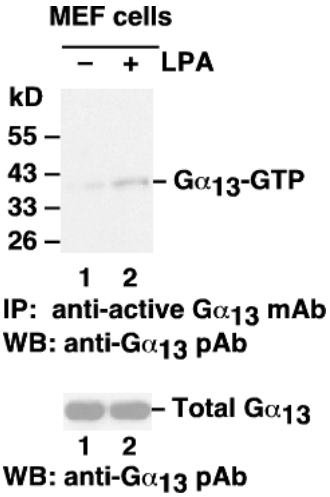
   *Note: If Nitrocellulose is used instead of PVDF, this step should be skipped.*

2. Block the membrane with 5% non-fat dry milk or 3% BSA in TBST for 1 hr at room temperature with constant agitation.

   Incubate the membrane with anti-$G\alpha_{13}$ polyclonal antibody, freshly diluted 1:100 in 5% non-fat dry milk or 3% BSA/TBST, for 1-2 hr at room temperature with constant agitation or at $4^{o}C$ overnight.
3. Wash the blotted membrane three times with TBST, 5 minutes each time.
4. Incubate the membrane with a secondary antibody (e.g. Goat Anti-Rabbit IgG, HRP-conjugate), freshly diluted 1:1000 in 5% non-fat dry milk or 3% BSA/TBST, for 1 hr at room temperature with constant agitation.
5. Wash the blotted membrane three times with TBST, 5 minutes each time.
6. Use the detection method of your choice such as ECL.

## Example of Results

The following figure demonstrates typical results seen with NewEast Biosciences $G\alpha_{13}$ Activation Assay Kit. One should use the data below for reference only.




$G\alpha_{13}$ activation assay. MEF cells were treated with (lane 2) or without (lane 1) LPA. Cell lysates were incubated with an anti-active $G\alpha_{13}$ monoclonal antibody (Cat. # 26902) (top panel). The precipitated active $G\alpha_{13}$ was immunoblotted with an anti- $G\alpha_{13}$ rabbit polyclonal antibody (Cat # 21005). The bottom panel shows the Western blot with anti- $G\alpha_{13}$ of the cell lysates used (5% of that used in the top panel).