
Product Manual

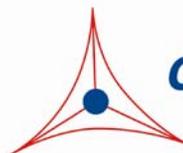
Active Rac-GEF Assay Kit (Tiam1)

Catalog Number

STA-422

20 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Small GTP-binding proteins (or GTPases) are a family of proteins that serve as molecular regulators in signal transduction pathways. Rac, a 21 kDa protein, belongs to the family of Rho GTPases regulating a variety of biological response pathways that include cell motility, cell division, gene transcription, and cell transformation. Like other small GTPases, Rac influences molecular events by cycling between an inactive GDP-bound form and an active GTP-bound form. Cycling between the GDP-bound and GTP-bound state is regulated primarily by two distinct families of proteins: guanine nucleotide exchange factors (GEFs) activate Rho proteins by catalyzing the exchange of GDP for GTP, the GTPase activating proteins or GAPs negatively regulate GTPase function by stimulating GTP hydrolysis (Figure 1).

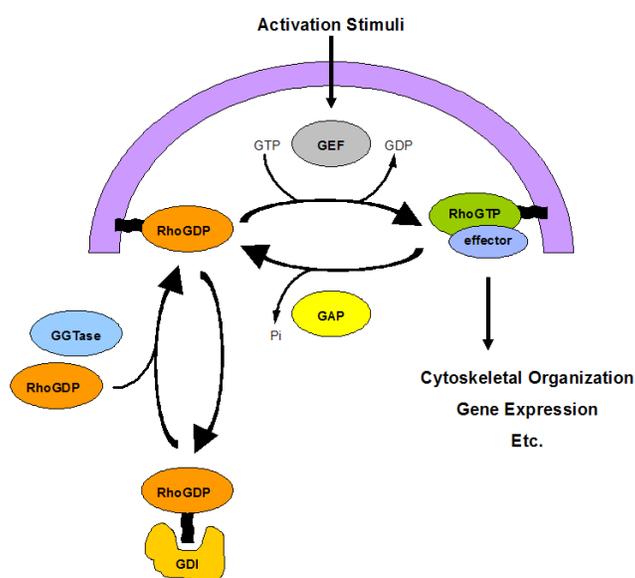


Figure 1. The Rho GTPase Switch

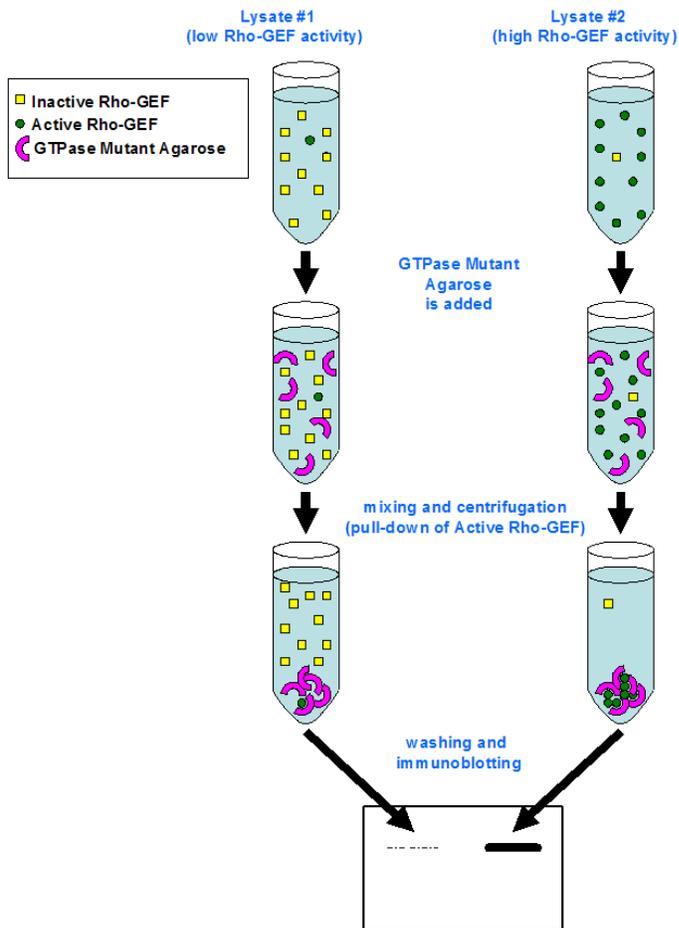
Similar to Ras mutants, constitutively active or dominant negative Rho GTPase mutants have been used to bind to Rho-GAP and effectors or to Rho-GEFs, respectively. A nucleotide-free GTPase has also been shown to form a high affinity binary complex with Rho-GEFs. Cell Biolabs Active Rac-GEF Assay Kit utilizes nucleotide-free Rac1 G15A Agarose beads to selectively isolate and pull-down the active form of Rac-GEFs from purified samples or endogenous lysates. Subsequently, the precipitated active Rac-GEF is detected by western blot analysis using an anti-Rac-GEF antibody (see Assay Principle below).

Cell Biolabs' Active Rac-GEF Assay Kit provides a simple and fast tool to monitor the activation of Rac-GEF. The kit includes easily identifiable Rac1 G15A Agarose beads (Figure 2), pink in color and anti-Tiam1 antibody. Each kit provides sufficient quantities to perform 20 assays.



Figure 2: Rac1 G15A Agarose beads, in color, are easy to visualize, minimizing potential loss during washes and aspirations.

Assay Principle



Related Products

1. STA-400: Pan-Ras Activation Assay Kit
2. STA-400-H: H-Ras Activation Assay Kit
3. STA-400-K: K-Ras Activation Assay Kit
4. STA-400-N: N-Ras Activation Assay Kit
5. STA-401: Rac Activation Assay Kit
6. STA-402: Cdc42 Activation Assay Kit
7. STA-403: Rho Activation Assay Kit
8. STA-404: Rac/Cdc42 Activation Assay Kit
9. STA-405: Rho/Rac/Cdc42 Activation Assay Kit
10. STA-431: RhoA G17A Agarose Beads (Active Rho-GEF)
11. STA-432: Rac1 G15A Agarose Beads
12. STA-433: Cdc42 G15A Agarose Beads

Kit Components

1. Rac1 G15A Agarose (Part No. STA-432): One vial – 800 μ L of 50% slurry, 1 mg/mL Rac1 G15A in PBS containing 50% glycerol.
Note: Agarose bead appears pink in color for easy identification, washing, and aspiration.
2. 5X Assay/Lysis Buffer (Part No. 242002): One bottle – 30 mL of 100 mM HEPES, pH 7.5, 750 mM NaCl, 5% Triton X-100, 25 mM MgCl₂.
3. Anti-Tiam1, Rabbit Polyclonal (Part No. 242202): One vial – 40 μ L in PBS, pH 7.4, 0.05% NaN₃, 0.1% BSA. The epitope recognized by the Anti-Tiam1 antibody is mapped to the C-terminal region of Tiam1 between residues 1550 and 1591.

Materials Not Supplied

1. Stimulated and non-stimulated cell lysates
2. Protease inhibitors
3. 4°C tube rocker or shaker
4. 2X reducing SDS-PAGE sample buffer
5. Electrophoresis and immunoblotting systems
6. Immunoblotting wash buffer such as TBST (10 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 0.05% Tween-20)
7. Immunoblotting blocking buffer (TBST containing 5% Non-fat Dry Milk)
8. PVDF or nitrocellulose membrane
9. Secondary Antibody
10. ECL Detection Reagents

Storage

Store all kit components at -20°C. Avoid multiple freeze/thaw cycles.

Preparation of Reagents

- 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.

Preparation of Samples

I. 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 (14,000 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.

II. 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 1×10^7 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 (14,000 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 Protocol

I. Active Rac-GEF 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. Thoroughly resuspend the Rac1 G15A Agarose bead slurry by vortexing or titrating.
4. Quickly add 40 µL of resuspended bead slurry to each tube.
5. Incubate the tubes at 4°C for 1 hour with gentle agitation.
6. Pellet the beads by centrifugation for 10 seconds at 14,000 x g.
7. Aspirate and discard the supernatant, making sure not to disturb/remove the bead pellet.
8. Wash the bead 3 times with 0.5 mL of 1X Assay/Lysis Buffer, centrifuging and aspirating each time.
9. After the last wash, pellet the beads and carefully remove all the supernatant.
10. Resuspend the bead pellet in 40 µL of 2X reducing SDS-PAGE sample buffer.
11. Boil each sample for 5 minutes.
12. Centrifuge each sample for 10 seconds at 14,000 x g.

II. Electrophoresis and Transfer

1. Load 20 µL/well of pull-down supernatant to a polyacrylamide gel. 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 as per the manufacturer's instructions.
3. Transfer the gel proteins to a PVDF or nitrocellulose membrane as per 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 in TBST for 1 hr at room temperature with constant agitation.

Incubate the membrane with Anti-Tiam1 Antibody, freshly diluted 1:1000 in 5% non-fat dry milk/TBST, for 1-2 hr at room temperature with constant agitation.

Note: To conserve antibody, incubations should be performed in a plastic bag.

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 in 5% non-fat dry milk/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. We recommend enhanced chemiluminescence reagents from Pierce.

Example of Results

The following figure demonstrates typical results seen with Cell Biolabs Active Rac-GEF Assay Kit (Tiam1). One should use the data below for reference only.

Figure 3: Tiam1 Activation Assay. 293 cells were transfected with active Tiam1 (Δ N Tiam1). Active Tiam1 in lysate was pulled down with Rac1 G15A agarose beads. *Lane 1*, Mock Transfection Control. *Lane 2*, Δ N Tiam1 Transfection.



Figure 4: Tiam1 Activation Assay in MDA-231 Cells. Active Tiam-1 in 2 mg of MDA-231 lysate was pulled down with Rac1 G15A agarose beads and probed with anti-Tiam1 antibody according to the Assay Protocol.



References

1. Arthur, W.T., Ellenbroek, S.M., Der, C.J., and Burridge K. (2002) *J. Biol. Chem.* **277**, 42964-42972
2. Garcia-Mata R., Wennerberg, K., Arthur, W.T., Noren, N.K., Ellenbroek, S.M., and Burridge K. (2006) *Methods Enzymol.* **406**, 425-437.
3. Habets, G.G., Scholtes, E.H., Zuydgeest, D., van der Kammen, R.A., Stam, J.C., Berns, A. and Collard, J.G. (1994) *Cell.* **77**, 537-549.

Recent Product Citation

1. Wang, P. et al. (2015). Selective killing of K-ras–transformed pancreatic cancer cells by targeting NAD (P) H oxidase. *Chin J Cancer.* **34**:1-11.
2. Oubaha, M. et al. (2012). Formation of a PKC/ β -catenin complex in endothelial cells promotes angiopoietin-1–induced collective directional migration and angiogenic sprouting. *Blood.* **120**: 3371-3381.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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