A helping hand for your research

Configuration-specific Monoclonal Antibody Based

Arl1 Activation Assay Kit

(30 Assays)

Cat. # 82901

FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC APPLICATIONS

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Arl1 Activation Assay Kit Protocol
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Introduction

A. Background

Arf-like protein 1 (Arl1) is a member of the Arf family of regulatory GTPases, within the Ras superfamily of GTPases, and with highly conserved orthologs throughout eukaryotes. Arl1 is essential for early embryonic development in Drosophila and in Caenorhabditis elegans. Arl1 is most similar in primary sequence, cellular location, and function (regulation of membrane traffic) to Arf1–6 and even shares several common binding partners. In addition to its function in membrane traffic at the Golgi/trans-Golgi network, there are reports indicating a possible role for Arl1 in ion homeostasis in yeast.

Currently there is no direct assay to measure the activation of Arl1 GTPases.

NewEast Biosciences Arl1 Activation Assay Kit is based on the configuration-specific monoclonal antibody that specifically recognizes Arl1-GTP, but not Arl1-GDP. Given the high affinity of monoclonal antibodies to their antigens, the activation assay could be performed in a short time. This assay provides the reliable results with consistent reproducibility.

B. Assay Principle

NewEast Biosciences Arl1 Activation Assay Kit uses configuration-specific anti-Arl1-GTP Mouse monoclonal antibody to measure Arl1-GTP levels in cell extracts or in vitro GTPγS loading Arl1 activation assays. Anti-Arl1-GTP mouse monoclonal antibody is first incubated with cell lysates containing Arl1-GTP. Next, the GTP-bound Arl1 is pulled down by protein A/G agarose. Finally, the precipitated Arl1-GTP is detected through immunoblot analysis using anti-Arl1 mouse monoclonal antibody.

C. Kit Contents

This kit contains enough reagents for approximately 30-35 pull-down assays.

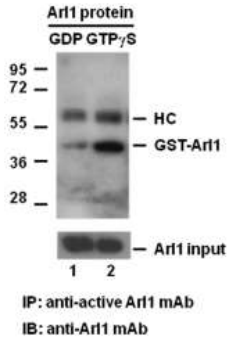
Reagent	Cat. #	Quantity	Storage
Anti-Arl1-GTP Mouse Monoclonal Antibody	26924	35 µL	-20°C
Protein A/G Agarose	30301	600 µL	4°C
5X Assay/Lysis Buffer	30303	30 mL	4°C
Anti-Arl1 Mouse Monoclonal Antibody	26056	50 µL	-20°C
100X GTPγS	30302	50 µL	-20°C
100X GDP	30304	50 µL	-20°C
HRP-Goat Anti-Rabbit IgG	29002	50 µL	-20°C

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 Arl1 Activation Assay Kit. For reference only.



Arl1 Activation Assay. Purified GST-tagged Arl1 proteins were immunoprecipitated with the anti-Arl1-GTP monoclonal antibody (Cat. # 26924) after treated with GDP (lane 1) or GTPγS (lane 2), and was blotted with anti-Arl1 monoclonal antibody (Cat. # 26056). Arl1 protein input control is shown in the bottom panel.

Assay Procedure

A. Reagent Preparation

1X Assay/Lysis Buffer: Mix the 5X Stock (Cat. # 30301) briefly and dilute to 1X in deionized water. Just prior to usage, add protease inhibitors such as 1 mM PMSF, 10 µg/mL leupeptin, or 10 µg/mL aprotinin.

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B. Sample Preparation

Adherent Cells

1. Culture cells (one 10-cm plate, ~10^7 cells) to approximately 80-90% confluence. Stimulate the 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 (See Reagent Preparation) to the cells (0.5–1 mL per 10 cm 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 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 centrifuging at 12,000 x g and 4°C for 10 minutes.
9. Collect the supernatant and store the sample (~1–2 mg of total protein) 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 through 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 (See Reagent Preparation) to the cell pellet (0.5-1 mL per 10^7 cells).
5. Lyse the cells by repeated pipetting.
6. Transfer the lysates to appropriate size tubes and place them on ice.
7. If nuclear lysis occurs, the cell lysates may become 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 centrifuging at 12,000 x g and 4°C for 10 minutes.
9. Collect the supernatant and store sample on ice for immediate use, or snap freeze and store at -70°C for future use.

C. In vitro GTPγS/GDP Protein for Positive and Negative controls

Note: In vivo stimulation of cells will activate approximately 10% of the available Arl1, whereas in vitro GTPγS protein loading will activate nearly 90% of Arl1.

1. Aliquot 0.5 mL of cell extract (or 1 µg of purified Arl1 protein) into two microcentrifuge tubes.
2. To each tube, add 20 µL of 0.5 M EDTA (final concentration of 20 mM).
3. Add 5 µL of 100 X GTPγS (Cat. # 30302) to the first tube as a positive control.
4. Add 5 µL of 100 X GDP (Cat. # 30304) to the second tube as a negative control.
5. Incubate both tubes at 30°C for 30 minutes with agitation.
6. Stop loading by placing the tubes on ice and adding 32.5 µL of 1 M $MgCl_2$ (final concentration of 60 mM).

D. Affinity Precipitation of Activated G Protein

1. Aliquot 0.5-1 mL of cell lysates (about 1 mg of total cellular protein) to a microcentrifuge tube.
2. Adjust the volume to 1 mL with 1X Assay/LysisBuffer (See Reagent Preparation).
3. Add 1 µL anti-Arl1-GTP antibody (Cat. # 26924).
4. Prepare the protein A/G Agarose bead slurry (Cat. # 30301) by resuspending through vertexing or titrating.
5. Quickly add 20 µL of resuspended bead slurry to above tube.
6. Incubate the tube at 4°C for 1 hour with gentle agitation.
7. Pellet the beads through centrifugation at 5,000 x g for 1 min.
8. Aspirate and discard the supernatant (making sure not to disturb or remove the bead pellet.
9. Wash the beads 3 times with 0.5 mL of 1X Assay/Lysis Buffer, centrifuging and aspirating each time.
10. After the third wash, pellet the beads through centrifugation and carefully remove all the supernatant.
11. Resuspend the bead pellet in 20 µL of 2X reducing SDS-PAGE sample buffer.
12. Boil the sample for 5 minutes.
13. Centrifuge it at 5,000 x g for 10 seconds.

E. Western Blot Analysis

1. Load 15 µL/well of pull-down supernatant to a polyacrylamide gel (17%). It is recommended to include a pre-stained MW standard (as an indicator of a successful transfer in step 3 below).
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.

Note: Steps 4-11 are at room temperature with agitation

4. Following electroblotting, 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, step 4 Should be skipped.

5. Block the membrane with 5% non-fat dry milk or 3% BSA in TBST for 1 hr at room temperature with constant agitation.
6. Wash the blotted membrane three times with TBST, 5 minutes each time.
7. Incubate the membrane with anti-Arl1 Mouse Monoclonal Antibody (Cat. # 26056), which is freshly diluted 1:50~500 (depending on the amount of Arl1 proteins in your sample) in 5% non-fat dry milk or 3% BSA in TBST, for 1-2 hr at room temperature with constant agitation or at 4°C overnight.
8. Wash the blotted membrane three times with TBST, 5 minutes each time.
9. Incubate the membrane with a secondary antibody (Cat. # 29002), which is freshly diluted 1:1000 in 5% non-fat dry milk or 3% BSA in TBST, for 1 hr at room temperature with constant agitation.
10. Wash the blotted membrane three times with TBST, 5 minutes each time.
11. Use the detection method of your choice such as ECL.