

RhoA (Mature form), Active

Human recombinant protein expressed in E.coli cells

Catalog # R144-310H

Lot # B2166-11

Product Description

Recombinant human RhoA (1-190) was expressed in E.coli cells using an N-terminal His tag. The gene accession number is [NM_001664](#).

Gene Aliases

ARH12; ARHA; RHO12; RHOH12

Formulation

Recombinant protein stored in 50mM sodium phosphate, pH 7.0, 300mM NaCl, 150mM imidazole, 0.1mM PMSF, 0.25mM DTT, 25% glycerol.

Storage and Stability

Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

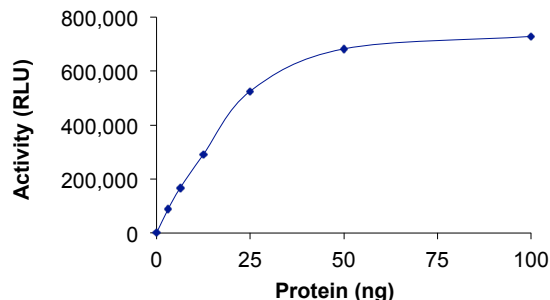
Scientific Background

RhoA is a member of the Rho gene family that interacts with protein kinases and serves as a target of activated GTPase. RhoA plays an important role in the initial events of cell protrusion, whereas Rac1 and Cdc42 activate pathways implicated in reinforcement and stabilization of newly expanded protrusions. Rho mediates corneal epithelial migration in response to external stimuli by regulating the organization of the actin cytoskeleton (1). The inhibition of RhoA signaling is required for both vaccinia morphogenesis and virus-induced cell motility. Vaccinia inhibits RhoA signalling using the viral protein F11 and this can enhance the spread of infection (2).

References

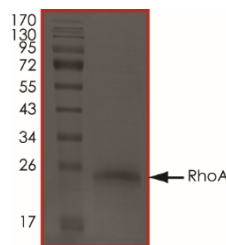
1. Wu, K. Y.et.al: Local translation of RhoA regulates growth cone collapse. Nature 436: 1020-1024, 2005.
2. Valderrama, F.et.al: Vaccinia virus-induced cell motility requires F11L-mediated inhibition of RhoA signaling. Science 311: 377-381, 2006.

Specific Activity



The specific activity of RhoA was determined to be **10.9 nmol/min/mg** as per activity assay protocol.

Purity



The purity of RhoA was determined to be **>85%** by densitometry. Approx. MW **23kDa**.

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Catalog Number	R144-310H
Specific Activity	10.9 nmol/min/mg
Lot #	B2166-11
Purity	>85%
Concentration	0.1 µg/µl
Stability	1yr at -70°C from date of shipment
Storage & Shipping	Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at the recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active RhoA (Catalog #: R144-310H)

Active RhoA (0.1µg/µl) diluted with GTPase/GAP Buffer and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active RhoA for optimal results).

GTPase-Glo™ Assay (Promega, Catalog# V7681)

GTPase/GAP Buffer, 5ml
GEF Buffer, 5ml
GTPase-Glo™ Buffer, 5ml
GTPase-Glo™ Reagent, 500X, 15µl
ADP, 10mM, 0.5ml
Detection Reagent, 10ml
rGTP, 10mM, 50µl
DTT, 100mM, 0.1ml

Assay Protocol

The GTPase assay is performed using the GTPase-Glo™ Assay kit (Promega), by detecting the amount of GTP remaining after GTP hydrolysis in a GTPase reaction. The remaining GTP is converted to ATP using the GTPase-Glo™ Reagent, and the ATP is then detected using a thermostable luciferase and luciferin substrate to produce bioluminescence. GTPase activity is inversely correlated to the amount of light produced.

Step 1. Thaw the active RhoA on ice and prepare the following working solutions with GTPase/GAP Buffer:

- 2X final concentration of Active RhoA (Catalog #: R144-310H)
- 2X GTP solution containing 2µM GTP and 1mM DTT

Step 2. In a polystyrene 96-well plate, add the following components to bring the initial reaction volume to 20 µl:

Component 1.	10 µl of 2X Active RhoA (Catalog #: R144-310H)
Component 2.	10 µl of 2X GTP solution

Note: A blank control can be set up as outlined in step 2 by replacing the enzyme working solution with an equal volume of GTPase/GAP Buffer.

- Step 3.** Mix the reaction on an orbital shaker for 2 minutes. Incubate the reaction at room temperature for the optimal time, generally 60 minutes.
- Step 4.** Prepare the required volume of reconstituted GTPase-Glo™ Reagent (1X) containing 5µM ADP with GTPase-Glo™ Buffer, equilibrate to room temperature before use.
- Step 5.** Add 20µl of reconstituted GTPase-Glo™ Reagent to the completed GTPase reactions, mix briefly and incubate with shaking at room temperature for 30 minutes.
- Step 6.** Add 40µl of Detection Reagent and incubate the plate for 5-10 minutes at room temperature.
- Step 7.** Read the plate using the KinaseGlo Luminescence Protocol on a GloMax plate reader (Promega; Cat# E7031).
- Step 8.** Determine the corrected activity (RLU) by removing each sample's value from the blank control (see Step 2) and calculate the GTPase specific activity as outlined below.

Calculation of GTP Specific Activity (SA) (RLU/pmol)

Specific activity (SA) = RLU of the blank control / pmoles of GTP in the blank control
(i.e., 10µl * 2µM GTP * 10⁻⁶ = 20 µmols * 10⁻⁶ = 20 pmols)

GTPase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected RLU from reaction / [(SA of GTP in RLU/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]

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MATERIAL SAFETY DATA SHEET

Article 1 - Product Identification and Use

Product Name: RhoA (Mature form), Active

Catalog # R144-310H

This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.

Manufacturer's Name: SignalChem Pharmaceuticals Inc.
Street Address: 110-13120 Vanier Place
City, Prov. Postal Code: Richmond, BC, V6V 2J2
Fax: 604-232-4601
EMERGENCY PHONE: 604-232-4600

Article 2 - Hazardous Ingredients

NOT AVAILABLE. We are not aware of any hazards associated with this product or its ingredients, but the chemical, physical, and toxicological properties of this product have not been investigated thoroughly. Observe normal laboratory precautions.

Article 3 - Physical Data

This product consists of purified protein in sodium phosphate buffer shipped on dry ice. The physical properties of this product have not been investigated thoroughly.

Article 4 - Fire and Explosion Hazard

NOT APPLICABLE

Article 5 - Reactivity Data

NOT APPLICABLE

Article 6 - Toxicologically Data

May be harmful by inhalation, ingestion, or skin absorption. The toxicological properties of this product have not been investigated thoroughly. Exercise due caution.

Article 7 - Preventative Measures

Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.

****MULTIPLE COMPONENT SPILL OR LEAK PROCEDURES****

- Wear protective equipment.
 - Absorb on sand or vermiculite and place in closed containers for disposal.
 - Observe all federal, state and local environmental regulations.
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Article 8 - First Aid Measures

- If swallowed, wash out mouth with water, provided person is conscious. Call a physician.
 - In case of skin contact, flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. If a rash or other irritation develops, call a physician.
 - If inhaled, remove to fresh air. If breathing becomes difficult, call a physician.
 - In case of eye contact, flush with copious amounts of water for at least 15 minutes while separating the eyelids with fingers. Call a physician.
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Article 9 - Preparation

Prepared By: Jun Yan

Phone #: 1-866-954-6273

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. SignalChem shall not be held liable for any damage resulting from handling or from contact with the above product. See the Technical Specification, Packing Slip, Invoice, and Product Catalog for additional terms and conditions of sale.

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