

## RhoC (Mature form), Active

Recombinant human protein expressed in E. coli cells

Catalog # R144-310CH

Lot # B2166-12

### Product Description

Recombinant human RhoC (1-190) was expressed in E. coli cells using an N-terminal His tag. The gene accession number is [NM\\_175744](#).

### Alternative Name(s)

ARH9; ARHC; H9; MGC1448; MGC61427; RHOH9

### 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.

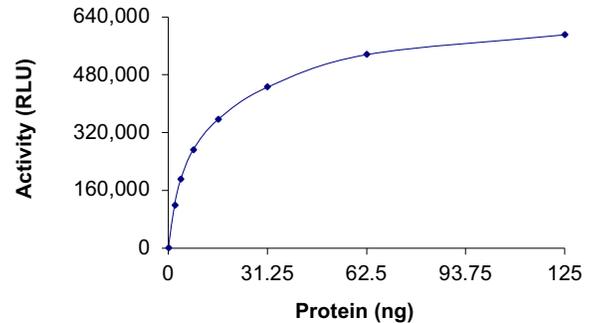
### Scientific Background

RhoC is member of the Rho family of small GTPases which promote reorganization of the actin cytoskeleton during cell morphogenesis and motility and regulate cell shape, attachment and motility. Active RhoC signals to its downstream effector ROCK which phosphorylates and activates LIM kinase that in turn phosphorylates cofilin inhibiting its actin-depolymerizing activity (1). Overexpression of RhoC is associated with tumor cell proliferation and metastasis while dominant-negative RhoC inhibits metastasis (2). Analysis of the phenotype of cells expressing dominant-negative RhoC indicates that RhoC is important in tumor cell invasion.

### References

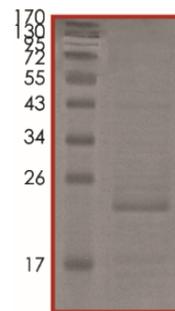
- Maekawa, M. et al: Signaling from Rho to the actin cytoskeleton through protein kinases ROCK and LIM-kinase. Science 285: 895-898, 1999.
- Clark, E. A. et al: Genomic analysis of metastasis reveals an essential role for RhoC. Nature 406: 532-535, 2000.

### Specific Activity



The specific activity of RhoC was determined to be **14.5 nmol/min/mg** as per activity assay protocol.

### Purity



The purity of RhoC was determined to be **>75%** by densitometry.  
Observed MW ~23 kDa.  
Calculated MW ~22 kDa.

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Recombinant human protein expressed in E. coli cells

Catalog #	R144-310CH
Specific Activity	14.5 nmol/min/mg
Lot #	B2166-12
Purity	>75%
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 RhoC (Catalog #: R144-310CH)

Active RhoC (0.1 µg/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 RhoC 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 RhoC 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 RhoC on ice and prepare the following working solutions with GTPase/GAP Buffer:

- 2X final concentration of Active RhoC
- 2X GTP solution containing 8 µM GTP and 2 mM DTT

**Step 2.** In a white opaque half-area 96-well plate, add the following components to bring the initial reaction volume to 20 µl:

**Component 1.** 10 µl of 2X Active RhoC

**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 1 minute. Incubate the reaction at 37°C for the optimal time, generally 60-90 minutes.

*Note: A series of rGTP standard solutions can be dispensed to the same plate at the end of the incubation period in order to determine the specific activity of the enzyme.*

**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 on a microplate luminometer.

**Step 8.** Using the GTP standard curve, determine the amount of GTP consumed (ΔGTP, µM) and calculate the enzyme specific activity as outlined below.

$$\text{Enzyme Specific Activity (SA) (nmol/min/mg)} \\ = \frac{[\Delta\text{GTP}] (\mu\text{M}) \times \text{Reaction Volume} (\mu\text{l})}{\text{Reaction Time (min)} \times \text{Enzyme Amount (mg)}} \times 10^{-3}$$

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# SAFETY DATA SHEET

## Article 1 – Product Identification

**Product Name: RhoC (Mature form), Active**

**Catalog # R144-310CH**

*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 Biotech 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 - Hazard Identification

- WHMIS Classification:** Not WHMIS controlled.
- GHS classification:** Skin irritation (Category 3); Eye irritation (Category 2B).
- Hazard Pictograms:** none.
- Signal words:** Warning.
- Hazard statements:** Causes mild skin irritation (H316); Causes eye irritation (H320).
- Precautionary statements:** IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. (P305 + P351 + P338).
- Other hazards:** none known.

## Article 3 – Composition/Information on Ingredients

**Chemical Characterization:** Mixtures.

**Description:** This product consists of the substances listed below.

Common name	Chemical name	CAS-No.	Concentration
Glycerol	Glycerol	56-81-5	25%
NaCl	Sodium chloride	7647-14-5	1.75%
Imidazole	1,3-Diaza-2,4-cyclopentadiene	288-32-4	≤1.02%
Sodium Phosphate, Dibasic	Sodium Phosphate, Dibasic	7782-85-6	1.34%
Protein		No data available	≤0.02%
DTT; Dithiothreitol	(R*,R*)-1,4-Dimercaptobutane-2,3-diol	3483-12-3	0.0038%
PMSF; Phenylmethanesulfonyl fluoride	α-Toluenesulphonyl fluoride	329-98-6	0.002%

## Article 4 – First-aid Measures

- General information:** Consult a physician by providing the SDS.
- After inhalation:** Breathe in fresh air. If cannot breathe, give artificial respiration and consult a physician.
- After skin contact:** Immediately wash with soap and plenty of water and rinse thoroughly. Consult a physician.
- After eye contact:** Rinse opened eyes with plenty of water for at least 15 minutes. Consult a physician.
- After swallowing:** rinse the mouth with plenty of water and consult a physician.

## Article 5 - Fire-fighting Measures

- Suitable extinguishing media:** Use water spray, extinguishing powder, carbon dioxide, or other appropriate measure that is suitable to the environment.
- Specific hazards arising from the substance or mixture:** None known.
- Special protective equipment and precautions for fire-fighters:** Self-contained breathing apparatus if necessary.

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## Article 6 – Accidental Release Measures

- Personal precautions, protective equipment and emergency procedures:** Apply standard laboratory practices and personal protective equipment. Avoid breathing vapors, mist, or gas. Ensure adequate ventilation.
- Environmental precautions:** Do not allow to enter drains.
- Methods and materials for containment and cleaning up:** Absorb on sand or vermiculite and place in closed containers for disposal.

## Article 7 - Handling and Storage

- Precautions for safe handling:** Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.
- Conditions for safe storage:** Store in a dry and well-ventilated place in -70 °C. Keep container upright and tightly closed.

## Article 8 - Exposure Controls/Personal Protection

- Components with limit monitoring values at workplace:**  
Glycerol (CAS-No: 56-81-5)

Values	Control parameters	Regulations
TWA	10 mg/m <sup>3</sup> for mist	British Columbia, Canada
TWA	3 mg/m <sup>3</sup> for respirable mist	British Columbia, Canada
TWA	10 mg/m <sup>3</sup>	Alberta, Canada
TWAEV	10 mg/m <sup>3</sup>	Ontario, Canada
TWAEV	10 mg/m <sup>3</sup>	Quebec, Canada
TWA	10 mg/m <sup>3</sup>	USA

- Appropriate engineering controls:**  
Apply adequate ventilation including mechanical exhaust or laboratory fume hood. Follow standard laboratory practices.
- Individual protection measures:**  
**Respiratory protection:**  
Use appropriate respirator if there is inadequate ventilation by following the government standards.  
**Hand protection:**  
Wear gloves and use proper glove removal technique to avoid skin contact. Discard gloves after use by following the applicable laboratory regulations. Wash and dry hands.  
**Eye/face protection:**  
Safety goggles with side-shields approved under appropriate government standards.  
**Skin/body protection:**  
Use appropriate clothing, footwear and any additional protection measures to protect from splashing or contamination.

## Article 9 – Physical and Chemical Properties

<b>Appearance:</b> Colorless fluid.	<b>Danger of explosion:</b> Product does not present an explosion hazard.
<b>Odour/Odour Threshold:</b> Not determined.	<b>Explosion limits:</b> Lower: 0.9 Vol %; Upper: 0.0 Vol %.
<b>pH:</b> Not available.	<b>Decomposition temperature:</b> Not available.
<b>Melting point/freezing point:</b> Not determined.	<b>Vapor pressure at 20 °C:</b> 0.1 hPa
<b>Boiling point/Boiling range:</b> 100 °C.	<b>Density:</b> Not determined.
<b>Flash point:</b> > 100 °C.	<b>Relative density:</b> Not determined.
<b>Flammability (solid, gaseous):</b> Not determined.	<b>Vapor density:</b> Not determined.
<b>Ignition temperature:</b> 400 °C.	<b>Evaporation rate:</b> Not determined.
<b>Auto-igniting:</b> Product is not self-igniting.	<b>Solubility in / Miscibility with Water:</b> Fully miscible.

## Article 10 - Stability and Reactivity

- Reactivity:** Stable under recommended transport and storage conditions.
- Chemical stability:** Stable under recommended transport and storage conditions.
- Possible hazardous reactions:** No dangerous reactions known.
- Conditions to avoid:** Heat and moisture.
- Incompatible materials:** Strong acids/bases, strong oxidizing/reducing agents.
- Hazardous decomposition products:** Carbon oxides may formed under fire conditions; no known decomposition information for other decomposition products.

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## Article 11 - Toxicological Information

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- Acute toxicity:** Not available.
- LD/LC50:** Not available.
- Skin corrosion/irritation:** Not available.
- Serious eye damage/eye irritation:** Not available.
- Respiratory or skin sensitization:** Not available.
- Germ cell mutagenicity:** Not available.
- Carcinogenicity:** No components are listed in IARC, or NTP, or OSHA, or ACGIH.
- Reproductive toxicity:** Not available.
- Teratogenicity:** Not available.
- Specific target organ toxicity - single exposure/ - repeated exposure (GHS):** Not available.
- Aspiration hazard:** Not available.
- Potential health effects:**
  - Inhalation:** May be harmful if inhaled. May cause respiratory tract irritation.
  - Ingestion:** May be harmful if swallowed.
  - Skin:** May be harmful if absorbed through skin. May cause skin irritation.
  - Eyes:** May cause eye irritation.
- Signs and Symptoms of Exposure:**
  - Prolonged or repeated exposure can cause: Nausea, Dizziness.
- Synergistic effects:** Not available.

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## Article 12 - Ecological Information

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- Eco-toxicity:** Not applicable.
- Biodegradability:** Not applicable.
- Bio-accumulative potential:** Not applicable.
- Mobility in soil:** Not applicable.
- PBT and vPvB assessment:** Not applicable.
- Other adverse effects:** Not applicable.

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## Article 13 - Disposal Considerations

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- Disposal methods:** In accordance to applicable national, regional, or local laws and regulations. For additional handling information and protection of employees please refer to Article 7 and 8.
- Contaminated packaging:** Disposal should be made in accordance to official regulations. Use water or cleansing agents to clean the area.

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## Article 14 - Transport Information

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- DOT:** Not dangerous goods.
- IMDG:** Not dangerous goods.
- IATA:** Not dangerous goods.

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## Article 15 - Regulatory Information

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- WHMIS Classification:** Non-hazardous.
- GHS label elements:** Not applicable.
- Signal word:** Not applicable.
- Hazard statements:** Not applicable.

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## Article 16 - Other Information

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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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