SIGNALCHEM

Catalog #	Aliquot Size
R106-310H-05	5 µg
R106-310H-10	10 µg

KRAS (Mature form), Active

Recombinant human protein expressed in E. coli cells

Catalog # R106-310H

Lot # Q2321-4

Product Description

Recombinant human KRAS (2-186) was expressed in E. coli cells using an N-terminal His tag. The gene accession number is NM_033360.

Alternative Name(s)

C-K-RAS; CFC2; K-RAS2A; K-RAS2B; K-RAS4A; K-RAS4B; KI-RAS; KRAS1; KRAS2; NS; NS3; RALD; RASK2

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.

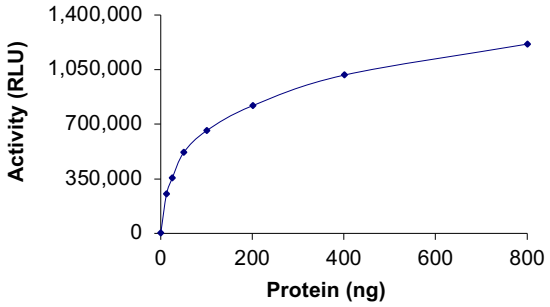
Scientific Background

The RAS gene superfamily encodes a group of closely related 21,000 dalton (p21) proteins with special affinity for guanine nucleotides (GTP). RAS and several other cellular proteins with similar biochemical properties are collectively known as G-proteins and they play key roles in a wide variety of cellular activities, including cell growth, differentiation, secretion, and protein trafficking (1). There are three forms of RAS gene in cells termed H-RAS, N-RAS, and K-RAS. RAS proteins play a direct causal role in human cancer and in other diseases. Mutant H-RAS, N-RAS, and K-RAS occur in varying frequencies in different tumor types (2). Other members of the RAS superfamily may also contribute to cancer.

References

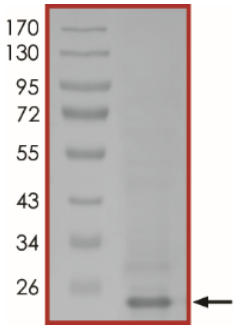
1. Shih, T. Y., et al: Structure and function of p21 ras proteins. Gene Amplif Anal. 1986;4:53-72.
2. Rodriguez-Viciana, P.: Cancer targets in the Ras pathway. Cold Spring Harb Symp Quant Biol. 2005;70:461-7.

Specific Activity



The specific activity of KRAS was determined to be **2.5 nmol/min/mg** as per activity assay protocol.

Purity



The purity of KRAS was determined to be **>80%** by densitometry.
Observed MW **~21 kDa**.
Calculated MW **~21 kDa**.

KRAS (Mature form), Active

Recombinant human protein expressed in E. coli cells

Catalog #	R106-310H
Specific Activity	2.5 nmol/min/mg
Lot #	Q2321-4
Purity	>80%
Concentration	0.1µg/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 KRAS (Catalog #: R106-310H)

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

- 2X final concentration of Active KRAS
- 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 KRAS

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.

Enzyme Specific Activity (SA) (nmol/min/mg)

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

SAFETY DATA SHEET

Article 1 – Product Identification

Product Name: KRAS (Mature form), Active **Catalog # R106-310H**

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 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	a-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 breath, 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 sate 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^3 for mist	British Columbia, Canada
TWA	3 mg/m^3 for respirable mist	British Columbia, Canada
TWA	10 mg/m^3	Alberta, Canada
TWAEV	10 mg/m^3	Ontario, Canada
TWAEV	10 mg/m^3	Quebec, Canada
TWA	10 mg/m^3	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

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

SAFETY DATA SHEET

Article 11 - Toxicological Information

- **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.

Article 12 - Ecological Information

- **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.

Article 13 - Disposal Considerations

- **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.

Article 14 - Transport Information

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

Article 15 – Regulatory Information

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

Article 16 - Other Information

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.