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Catalog #	Aliquot Size
R102-310H-05	5 µg
R102-310H-10	10 µg

RAC3, Active

Full-length human recombinant protein expressed in E.coli cells

Catalog # R102-310H
Lot # B2166-10

Product Description

Recombinant full length human RAC3 was expressed in E.coli cells using an N-terminal His tag. The gene accession number is NM_005052.

Gene Aliases

(None)

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.

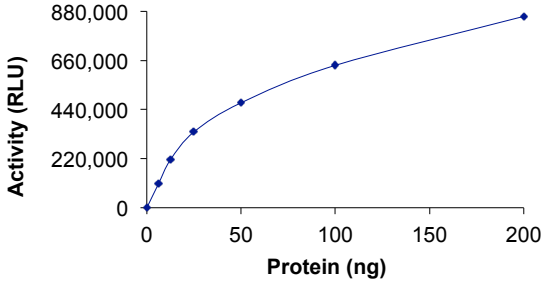
Scientific Background

RAC3 is a GTPase which belongs to the RHO family of small GTP-binding proteins that regulate a diverse array of cellular events, including the control of cell growth, cytoskeletal reorganization, and the activation of protein kinases. RAC3 is primarily expressed in brain and is proposed to play a specific function in neuronal cells. Depletion of RAC3 induces stronger cell adhesion and dramatically increases the outgrowth of neurite-like protrusions whereas overexpression of RAC3 results in a contractile round morphology (1). RAC3 can exert its function through negatively affecting integrin-mediated cell-matrix adhesions (2). RAC3 shares with RAC1 the ability to interfere with cadherin-mediated adhesion.

References

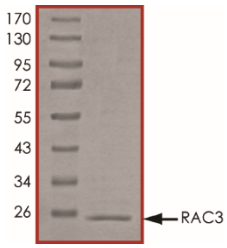
1. Hajdo-Milasinovic A, et al: Rac3 inhibits adhesion and differentiation of neuronal cells by modifying GIT1 downstream signaling. J Cell Sci. 2009 Jun 15;122(Pt 12):2127-36.
2. Lozano E, et al: PAK is required for the disruption of E-cadherin adhesion by the small GTPase Rac. J Cell Sci. 2008 Apr 1;121(Pt 7):933-8.

Specific Activity



The specific activity of RAC3 was determined to be **5.7 nmol/min/mg** as per activity assay protocol.

Purity



The purity of RAC3 was determined to be **>95%** by densitometry. Approx. MW **24 kDa**.

RAC3, Active

Full-length human recombinant protein expressed in E.coli cells

Catalog Number	R102-310H
Specific Activity	5.7 nmol/min/mg
Lot #	B2166-10
Purity	>95%
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 RAC3 (Catalog #: R102-310H)

Active RAC3 (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 RAC3 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 RAC3 on ice and prepare the following working solutions with GTPase/GAP Buffer:

- 2X final concentration of Active RAC3 (Catalog #: R102-310H)
- 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 RAC3 (Catalog #: R102-310H)
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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