

CDC42, Active

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

Catalog # C108-310GH

Lot # B2166-14

Product Description

Recombinant full length human CDC42 was expressed in E.coli cells using an N-dual GST/His tag. The gene accession number is [NM_001791](#).

Gene Aliases

G25K, CDC42Hs

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 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.

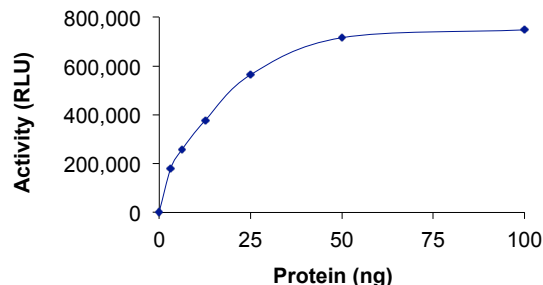
Scientific Background

CDC42 is a member of the Rho family of GTPases and is part of cellular pathways fundamental to growth, differentiation and apoptosis (1). Downstream targets for CDC42 include those that regulate the actin cytoskeleton (e.g. WASP) and cellular stress pathways (e.g. PAK) as well as the coatmer protein complex and PAR6. CDC42 is involved in the G(1)-S progression of the cell cycle. CDC42 and its downstream effector mDia3 are involved in bi-orientation and stabilization of spindle microtubules attachment to kinetochores and regulate chromosome alignment and segregation during mitosis (2).

References

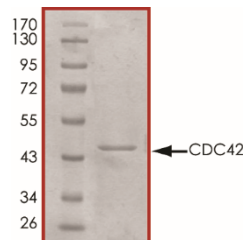
1. Erickson, J.W. et al: Multiple roles for Cdc42 in cell regulation. *Curr Opin Cell Biol.* 2001 Apr;13(2):153-7.
2. Narumya, S. et al: A new look at Rho GTPases in cell cycle: role in kinetochore-microtubule attachment. *Cell Cycle.* 2004 Jul;3(7):855-7.

Specific Activity



The specific activity of CDC42 was determined to be **17.5 nmol/min/mg** as per activity assay protocol.

Purity



The purity of CDC42 was determined to be **>95%** by densitometry. Approx. MW **48 kDa**.

CDC42, Active

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

Catalog Number	C108-310GH
Specific Activity	17.5 nmol/min/mg
Lot #	B2166-14
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 CDC42 (Catalog #: C108-310GH)

Active CDC42 (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 CDC42 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 CDC42 on ice and prepare the following working solutions with GTPase/GAP Buffer:

- 2X final concentration of Active CDC42 (Catalog #: C108-310GH)
- 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 CDC42 (Catalog #: C108-310GH)
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 µmoles * 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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