



**Cdc42-His Protein: wild-type
(Human recombinant)**

Cat. # CD01-XL Amount: 1 x 1 mg
Lot # 062 Store desiccated at -70°C

Material

The wild-type human Cdc42 protein has been produced in a bacterial expression system. The recombinant protein contains six histidine residues (His-tag) at its amino terminus. The approximate molecular weight of the His-Cdc42 protein is approximately 22 kDa. His-Cdc42 (1 mg of protein) is supplied as a lyophilized white powder.

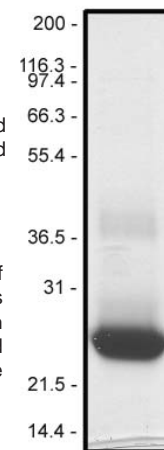
Storage and Reconstitution

Briefly centrifuge the tube to make sure that the lyophilized protein powder collects in the bottom of the tube. The protein should be reconstituted to 5 mg/ml by the addition of distilled water. The protein will be in the following buffer: 50 mM Tris pH 7.6, 0.5 mM MgCl₂, 50 mM NaCl, 3% sucrose, 0.6% dextran. In order to maintain high biological activity of the protein, it is strongly recommended that the protein solution be supplemented with DTT to 1 mM final concentration, aliquoted into "experiment sized" amounts and snap frozen in liquid nitrogen. The protein can be stored at -70°C for 6 months. The protein must not be exposed to repeated freeze-thaw cycles. The lyophilized protein is stable at 4°C for 1 year.

Purity

Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 12% polyacrylamide gradient gel. His-Cdc42 protein was determined to be 90% pure. (see Figure 1).

Figure 1. His-Cdc42 Protein Purity Determination. A 10 µg sample of recombinant His-Cdc42 protein (molecular weight approx. 22 kDa) was separated by electrophoresis in a 12% SDS-PAGE system and stained with Coomassie Blue. Protein quantitation was performed using the Precision Red Protein Assay Reagent (Cat.# ADV02). Mark12 molecular weight markers are from Invitrogen.



Biological Activity Assay

The biological activity of CD02 can be determined from its ability to exchange GTP. A standard biological assay for monitoring the GTP-bound form of Cdc42 consists of a pulldown assay using GST-tagged PAK-1 PBD beads (Cat. # PAK02) and GTP γ S or GDP loaded His-Cdc42. The PAK (p21 activated kinase CRIB domain) protein is an effector of Cdc42, and will specifically bind to the GTP bound form Cdc42. Using this assay, the amount of biologically active GTP-bound Cdc42 can be determined. Stringent quality control ensures that > 70% of the Cdc42 protein produced is capable of binding GTP.

Reagents

- 1) GST-tagged PAK-1 PBD protein on beads (Cat. # PAK02)
- 2) Recombinant Cdc42-His protein (Cat. # CD02)
- 3) Loading buffer (150 mM EDTA)
- 4) Stop buffer (600 mM MgCl₂)
- 5) Wash buffer (25 mM Tris pH 7.5, 30 mM MgCl₂, 40 mM NaCl)
- 6) Lysis buffer (10 mM Tris pH 7.5, 1 mM DTT, 25 mM NaCl)
- 7) GTP γ S (20 mM solution) (Cat. # BS01)
- 8) GDP (100 mM solution)
- 9) BSA (10 mg/ml)
- 10) Anti-Cdc42 polyclonal antibody (Cat. # ACD02)

Equipment

- 1) Microfuge at 4°C
- 2) SDS-PAGE and Western blot apparatus

Method

- 1) Dilute His-Cdc42 to 0.1 μ g/ μ l by the addition of ice cold lysis buffer.
- 2) Add 200 ng (2 μ l) of His-Cdc42 to 23 μ l Lysis buffer and 2 mM GTP γ S on ice.
- 3) Add 200 ng His-Cdc42 to 23 μ l Lysis buffer and 10 mM GDP on ice.
- 4) Add 1/10th the volume of loading buffer to each tube (final conc. 10 mM), and incubate at room temp. for 15 min.
- 5) Stop the reaction by adding 1/10th the volume of stop buffer to each tube (final conc. 60 mM)
- 6) Resuspend the PAK-1 PBD beads in water to 0.5 μ g/ μ l final protein concentration.
- 7) To each reaction tube add 215 μ l wash buffer, 10 μ g PAK-1 beads and 20 μ l 10 mg/ml BSA.
- 8) Gently rotate the tubes at 4°C for 1 h.
- 9) Centrifuge out the beads at 5k rpm in a microfuge at 4°C for 1 min.
- 10) Remove the supernatant and wash the beads in 1 ml of wash buffer. Remove the beads to a new tube
- 11) Centrifuge out the beads and resuspend the beads from each tube in 20 μ l of SDS sample buffer.
- 12) The bead and supernatant samples can now be analyzed by Western blot procedure using a Cdc42 specific polyclonal antibody (Cat. # ACD02).
- 13) Typical assay results are shown in Figure 2.

Figure 2. Selective Binding of GTP-bound His-Cdc42 to PAK02 Protein Beads *in vitro*.

His-Cdc42 was loaded with either GTP γ S (lane 2), or GDP (lane 3) as described in the method. The loaded proteins were incubated with 10 μ g of PAK-1 PBD protein beads, separated by centrifugation and subjected to Western blot analysis using a Cdc42 specific polyclonal antibody (Cat. # ACD02). Lane 1 shows 100 ng of recombinant His-Cdc42 control protein. SeeBlue molecular weight markers are from Invitrogen.

Product Uses

- * Measurement of the GTP/GDP ratio of Cdc42 *in vitro*.
- * Detection and quantitation of activated Cdc42 from tissue culture cell lysates
- * Identification of Cdc42 binding proteins
- * Study of Cdc42 function *in vivo* by the introduction of His-Cdc42 into live cells

Related Products and Uses

Cytoskeleton Inc. is the leading supplier of purified small G-proteins, visit our web site or call for information on the small G-proteins currently available. These include the small G-protein Activation Assay Kits, and a variety of affinity reagents for small G-protein activation assays:

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|--|--------------|
| * His-Cdc42 protein: Wild-type | Cat.# CD01 |
| * His-Cdc42 protein: constitutively active | Cat.# C6101 |
| * GST-Cdc42 protein: wild-type | Cat.# CDG01 |
| * GST-Cdc42 protein: wild-type | Cat.# CDG02 |
| * GST-Cdc42 protein: constitutively active | Cat.# C61G01 |
| * GST-Cdc42 protein: dominant negative | Cat.# C17G01 |
| * Cdc42 activation assay kit | Cat.# BK034 |

