V. 1.0

Cdc42-His Protein: wild-type
(Human recombinant)
Cat. # CD01

Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

Material

The wild-type human Cdc42 protein has been produced in a bacterial expression system. The recombinant protein contains a run of 6 histidine residues (His-tag) at its amino terminus. The approximate molecular weight of the Cdc42-His protein is approximately 22 kDa. Cdc42-His 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 Milli-Q water. The protein will be in the following buffer: 50 mM Tris pH 7.6, 0.5 mM $MgCl_2$, 50 mM NaCl, 3% sucrose and 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. Cdc42-His protein was determined to be 90% pure. (see Figure 1).

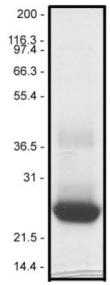


Figure 1. Cdc42-His Protein Purity Determination. A 10 µg sample of recombinant Cdc42-His tagged protein (molecular weight approx. 22 kDa) was separated by electrophoresis in a 12% SDS-PAGE system. The protein was stained with Coomassie Blue. Protein quantitation was performed using the Precision Red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

Biological Activity Assay

The biological activity of CD01 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 GTPγS or GDP loaded Cdc42-His. 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, aliquoted to 5 mg/ml and stored in 2 µl aliquots at -70°C (Cat. # CD01)
3. Loading buffer (150 mM EDTA)
4. Stop buffer (600 mM $MgCl_2$)
5. Wash buffer (25 mM Tris pH 7.5, 30 mM $MgCl_2$, 40 mM NaCl)
6. Lysis buffer (10 mM Tris pH 7.5, 1 mM DTT, 25 mM NaCl)
7. GTPγS (20 mM solution) (Cat. # BS01)
8. GDP (100 mM solution)
9. BSA (10 mg/ml)
10. Anti-Cdc42 monoclonal antibody (Cat.# ACD03)

Equipment

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

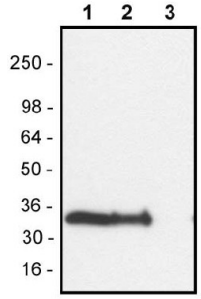
Method

1. Dilute Cdc42-His to 0.1 µg/µl by the addition of ice cold lysis buffer.
2. Add 200 ng (2 µl) of Cdc42-His to 23 µl Lysis buffer and 2 mM GTPγS on ice.
3. Add 200 ng Cdc42-His to 23 µl Lysis buffer and 10 mM GDP on ice.
4. Add $1/10^{th}$ 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 $1/10^{th}$ the volume of stop buffer to each tube (final conc. 60 mM)
6. Resuspend the PAK02 PBD beads in water to 0.5 µg/µl final protein concentration.
7. To each reaction tube add 215 µl wash buffer, 10 µg PAK02 beads, and 20 µl 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.

Phone: (303) 322.2254 **Fax:** (303) 322.2257
Customer Service: cserve@cytoskeleton.com
Technical Support: tservice@cytoskeleton.com

10. Remove the supernatant and wash the beads in 1ml of wash buffer. Remove the beads to a new tube.
11. Centrifuge out the beads and resuspend the beads from each tube in 20 µl of SDS sample buffer.
12. The bead and supernatant samples can now be analyzed by Western blot procedure using a Cdc42 specific monoclonal antibody (Cat.# ACD03). See ACD03 datasheet for specfic protocol.
13. Typical assay results are shown in Figure 2.

Figure 2. Selective Binding of PAK02 GST Tagged Protein



Beads to the GTP-bound Form of Cdc42 *In Vitro*.
Cdc42-His was loaded with either GTPγS (lane 2), or GDP (lane 3) as described in the method. The loaded proteins were incubated with 10 µg of PAK02-PBD GST tagged protein beads, separated by centrifugation and subjected to Western blot analysis using a Cdc42 specific monoclonal antibody (Cat. # ACD03). Lane 1 shows 100 ng of recombinant Cdc42-His 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 Cdc42-His into live cells

Product Citations/Related Products
For the latest citations and related products please visit www.cytoskeleton.com.