



**His-Cdc42 L61 Mutant Protein  
Constitutively Active**

**Cat. # C6101**

**Lot # 313                      Amount: 1 x 10 µg**

**Store at 4°C (desiccated) or at -70°C**

**Material**

The constitutively active form of the human Cdc42 protein has been produced in a bacterial expression system. The protein has a glutamine to leucine substitution at amino acid 61, creating a constitutively active mutant protein that will not hydrolyze GTP. The recombinant protein contains six histidine residues (His-tag) at its amino terminus. The approximate molecular weight of His-Cdc42 L61 protein is 25 kDa. His-Cdc42 L61 protein is supplied as a lyophilized white powder.

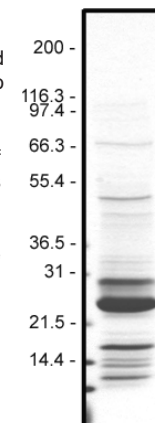
**Storage and Reconstitution**

The protein should be reconstituted to 1 mg/ml by the addition of 10 µl of distilled water. The protein will be in the following buffer; 10 mM Tris pH 7.5, 10 mM NaCl, 0.1 mM MgCl<sub>2</sub>, 0.5% sucrose and 0.1% 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, snap frozen in liquid nitrogen and stored at -70°C. The protein is stable for 6 months under these conditions. 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 gel. His-Cdc42 L61 protein was determined to be 70% pure (see Figure 1).

**Figure 1. His-Cdc42 L61 Protein Purity Determination.** A 10 µg sample of recombinant His-Cdc42 L61 protein (molecular weight approx. 25 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

His-Cdc42 L61 mutant protein can bind GTP but its intrinsic GTPase activity has been eliminated, resulting in a constitutively active protein. A standard biological assay for His-Cdc42 L61 activity consists of a pull-down assay using PAK-PBD beads (Cat. # PAK02). The PAK (p21 Activated Kinase CRIB domain) protein is an effector of Cdc42 and will specifically bind to active GTP-Cdc42. Stringent quality control ensures that > 80% of His-Cdc42 L61 protein can be pulled down using this method.

### Reagents

- 1) Recombinant His-Cdc42 L61 constitutively active protein (Cat. # C6101)
- 2) Recombinant His-Cdc42 wild-type protein (Cat. # CD01)
- 3) PAK-PBD beads (Cat. # PAK02)
- 4) Loading buffer (150 mM EDTA)
- 5) Stop buffer (600 mM MgCl<sub>2</sub>)
- 6) Wash buffer (25 mM Tris pH 7.5, 30 mM MgCl<sub>2</sub>, 40 mM NaCl)
- 7) Cell lysis buffer (50 mM Tris pH 7.5, 10 mM MgCl<sub>2</sub>, 0.3M NaCl, 2% IGEPAL)
- 8) GTP $\gamma$ S (20 mM solution) (Cat. # BS01)
- 9) GDP (100 mM solution)
- 10) BSA (10 mg/ml)
- 11) Anti-Cdc42 polyclonal antibody (Cat. # ACD02)

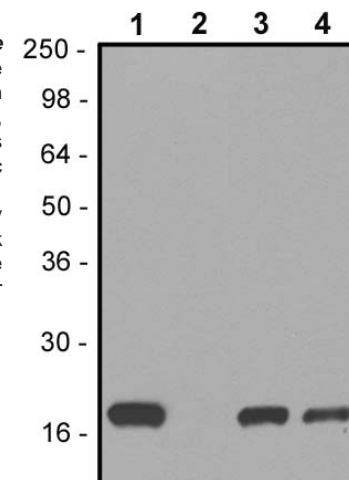
### Equipment

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

### Method

- 1) Dilute His-Cdc42 L61 constitutively active protein to 0.1  $\mu$ g/ $\mu$ l with cold Cell lysis buffer.
- 2) Dilute His-Cdc42 wild-type protein to 0.1  $\mu$ g/ $\mu$ l with cold Cell lysis buffer.
- 3) Resuspend PAK-PBD beads to 1  $\mu$ g/ $\mu$ l by the addition of 500  $\mu$ l distilled water.
- 4) Add 23  $\mu$ l of Cell lysis buffer and 2  $\mu$ l of Loading buffer into two microfuge tubes on ice.
- 5) Add 2  $\mu$ l (200 ng) of His-Cdc42 wild-type protein into both tubes.
- 6) Add 3  $\mu$ l of GTP $\gamma$ S to one tube and 3  $\mu$ l of GDP to the other tube. Incubate the loading reactions at room temperature for 15 min.
- 7) Repeat the nucleotide loading steps 4 through 6 with His-Cdc42 L61 constitutively active protein.
- 8) Stop all reactions with the addition of 4  $\mu$ l of Stop buffer and place on ice.
- 9) Add 215  $\mu$ l of cold Cell lysis buffer and 20  $\mu$ l of BSA to each tube on ice.
- 10) Add 10  $\mu$ l (10  $\mu$ g) of PAK-PBD beads to each tube and rotate for 30 min at 4°C
- 11) Pellet the beads at 8k rpm in a microfuge at 4°C for 1 min.
- 12) Remove the supernatant and wash the beads in 500  $\mu$ l of Wash buffer.
- 13) Pellet the beads as before and resuspend in 20  $\mu$ l of SDS sample buffer.
- 14) The bead and supernatant samples can now be analyzed by Western blot using a Cdc42 specific polyclonal antibody (Cat. # ACD02).
- 15) Typical assay results are shown in Figure 2.

**Figure 2. Binding of Wild-type and Constitutively active His-Cdc42 to PAK-PBD beads *in vitro*.** 200 ng of wild-type and constitutively active His-Cdc42 protein were loaded with either GTP $\gamma$ S (lanes 1 and 3) or GDP (lanes 2 and 4), subjected to a pull-down assay with 10  $\mu$ g of PAK-PBD beads and analyzed by Western blot using a Cdc42 specific polyclonal antibody as described in the method. Lanes 1 and 2, wild-type His-Cdc42. Lanes 3 and 4, constitutively active His-Cdc42. Note: GTP $\gamma$ S and GDP samples look identical in the constitutively active His-Cdc42 pull down due to extremely poor nucleotide exchange. SeeBlue molecular weight markers are from Invitrogen.



### Product Uses

- \* Identification of Cdc42 binding proteins
- \* Study of Cdc42 function *in vivo* by the introduction of constitutively active His-Cdc42 into live cells

### Related Products

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| * RhoA Activation Luminescence Assay, ELISA format       | Cat. # BK121  |
| * RhoA, B, C Activation Absorbance Assay, ELISA format   | Cat. # BK123  |
| * RhoA Activation Absorbance Assay, ELISA format         | Cat. # BK124  |
| * RhoGEF Exchange Assay kit                              | Cat. # BK100  |
| * RhoGAP Assay kit                                       | Cat. # BK105  |
| * Cdc42 Activation Assay                                 | Cat. # BK034  |
| * EasyRad Phosphate Assay (Radioactive based)            | Cat. # BK055  |
| * His-Cdc42 protein: wild-type                           | Cat. # CD01   |
| * GST-Cdc42 protein: wild-type                           | Cat. # CDG01  |
| * GST-Cdc42 protein: constitutively active               | Cat. # C61G01 |
| * GST-Cdc42 protein: dominant negative                   | Cat. # C17G01 |
| * GST-Cdc42 wild-type beads                              | Cat. # BC01   |
| * GST-Cdc42 constitutively active beads                  | Cat. # BC02   |
| * GST-Cdc42 dominant negative beads                      | Cat. # BC03   |
| * PAK-PBD beads  | Cat. # PAK02  |