

Cdc42-GST Protein

Wild-type

Cat. # CDG01

Upon arrival store at 4°C (desiccated)

See datasheet for storage after reconstitution

Material

The wild-type form of the human Cdc42 protein has been produced in a bacterial expression system. The recombinant protein is tagged with GST (28 kDa) at its amino terminus. The approximate molecular weight of the Cdc42-GST protein is 45 kDa. Cdc42-GST is supplied as a white lyophilized powder.

Storage and Reconstitution

Briefly centrifuge to collect the product at the bottom of the tube. The protein should be reconstituted to 1 mg/ml by the addition of 25 μ l of distilled water per tube. The protein will then be in the following buffer: 10 mM Tris pH 7.5, 20 mM NaCl, 0.3 mM MgCl₂, 1.0% sucrose and 0.2% 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 (10 μ g is recommended for one assay), snap frozen in liquid nitrogen and stored at -70°C. The protein is stable for 6 months if stored at -70°C. The protein should not be exposed to repeated freeze-thaw cycles. The lyophilized protein is stable for 1 year if stored desiccated to <10% humidity at 4°C.

Purity

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

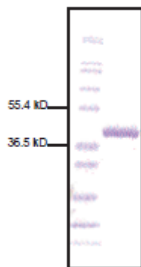


Figure 1. Cdc42-GST Protein Purity Determination. A 20 μ g sample of recombinant Cdc42-GST protein (molecular weight approx. 45 kDa) was separated by electrophoresis in a 12% SDS-PAGE system and stained with Coomassie Blue. Protein quantitation was performed using the Precision Red Protein Assay Reagent (Cat. # ADV02). Mark12 molecular weight markers are from Invitrogen.

Biological Activity Assay

Human Dbs is an exchange factor for Cdc42 and RhoA. The biological activity of Cdc42-GST can be determined through quantitation of the ability of hDbs to catalyze the exchange of GDP for GTP on Cdc42-GST. Stringent quality control ensures that the exchange rate of Dbs on Cdc42 is above 1.5 mmol mant-GTP/mol Cdc42/sec based on the standard assay protocol.

Reagents

1. Recombinant hDbs-His protein (Cat.# GE01)
2. Recombinant Cdc42-GST protein (Cat.# CDG01)
3. 2 x Exchange buffer (40 mM Tris pH 7.5, 300 mM NaCl, 20 mM MgCl₂, 2mM DTT, 1.5 μ M mant-GTP). Available in a user friendly Exchange Assay kit (Cat. # BK100).

Equipment

1. Fluorescence spectrometer (λ_{ex} =360nm, λ_{em} =440nm)
2. Corning 96-well half area plates (Cat # 3686) or other plate with low protein binding surface.

Method

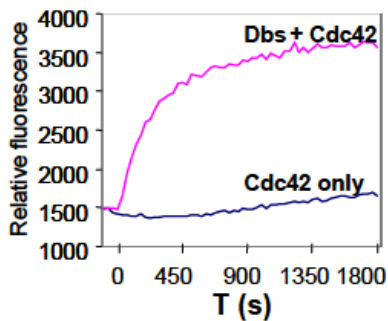
1. Dilute hDbs-His (Cat# GE01) to 0.3 μ g/ μ l (8 μ M).
2. Dilute Cdc42-GST (Cat# CDG01) to 2.5 mg/ml (50 μ M).
3. Dissolve exchange buffer in 5 ml milli-Q water and keep in room temperature.
4. Add the following components together and mix well by pipeting or gentle vortex:

Exchange reaction mix	96 well black plate
2x Exchange Buffer	50 μ l
50 μ l Cdc42	4 μ l
dH ₂ O	36 μ l

5. Aliquot to the assigned well and place the plate in the fluorimeter. Set up the fluorimeter (Excitation wavelength at 360 nm and emission wavelength at 440 nm) and start the reading.
6. After 5 cycles (150 seconds) pipette 10 μ l Dbs (8 μ M) protein or dH₂O in respective wells and immediately pipette up and down twice and resume reading for at least 30 minutes.
7. Save the readings after the kinetic protocols are finished. The exchange rate can be calculated by reducing the data to Vmax with the software that accompanies the plate reader. The exchange curve can be achieved by export to Microsoft Excel. Figure 2 shows typical exchange results for Cdc42-GST.

Figure 2. Cdc42 exchange assay.

CDG01 protein was mixed with exchange buffer and aliquoted to respective well in a 96-well half area plate. After 5 cycles of reading in a flurimeter, Dbs protein was added as described in the method.



Product Uses

- Identification of Cdc42 binding proteins.
- Characterization of signal transduction pathways involving Cdc42.

Product Citations/Related Products

For the latest citations and related products please visit www.cytoskeleton.com.