V. 1.0

hDbs-His Protein: DH/PH domain
(Human recombinant)
Cat. # GE01

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

Material

The human Dbs (DH/PH) 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 human Dbs-His (DH/PH) protein is approximately 40 kD. The protein is supplied as a lyophilized white powder.

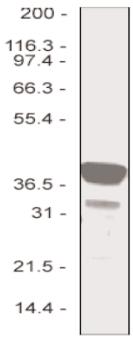
Storage and Reconstitution

The protein should be reconstituted to 2 mg/ml by the addition of 25 µl of distilled water. The protein will be in the following buffer; 20 mM Tris pH 7.5, 0.5 mM $MgCl_2$, 0.5% sucrose, 0.1% dextran. In order to maintain high biological activity of the protein, it is strongly recommended that the protein solution should be aliquoted into "experiment sized" aliquots and snap frozen in liquid nitrogen. The protein can be stored a -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 when stored desiccated (<10% humidity).

Purity

Protein purity is determined by scanning densitometry of Coomassie blue stained protein on a 4-20% polyacrylamide gradient gel. hDbs-His protein was determined to be >80% pure. (see Figure 1).

Figure 1. hDbs-His Protein Purity Determination. A 10 µg sample of recombinant Dbs-His tagged protein (molecular weight approx. 40 kD) was separated by electrophoresis in a 4-20% SDS-PAGE system. The protein was stained with coomassie blue. Protein quantitation was determined 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 hDbs can be determined from its ability to catalyze the exchange of GDP for GTP on Rho GTPases. A standard biological assay for monitoring the catalytic activity of Dbs is the exchange assay using RhoGEF exchange assay kit (Cat.# BK100). 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-His protein (Cat.# CD01)
3. Recombinant RhoA-His protein (Cat.# RH01)
4. 2 x Exchange buffer (40 mM Tris pH 7.5, 300 mM NaCl, 20 mM $MgCl_2$, 2mM DTT, 1.5 µM mant-GTP)

All reagents are available in biochemical kit Cat. # BK100.

Equipment

1. Fluorescence spectrometer (λex=360nm, λem=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 µg/ul (8 µM).
2. Dilute Cdc42-His (Cat# CD01) to 1.25 mg/ml (50 µM).
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 µl
50 µM Cdc42	4 µl
dH2O	36 µl

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 ul Dbs (8 µM) protein or dH_2O 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.

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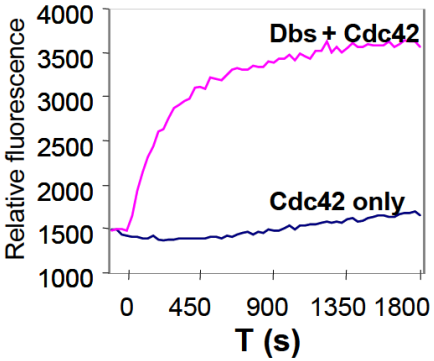
Figure 2. Cdc42 exchange assay.

Cdc42-His 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. Flurorescence data have been analyzed with respective software.



Product Uses

- Study of GEF activity with different GTPases.
- Identification of Dbs binding proteins
- Study of Dbs function in vivo by the introduction of Dbs- His into live cells
- Postive control for other RhoGEF studies.

Product Citations/Related Products

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