V. 1.0

RhoA-GST Protein
Wild-type
Cat. # RHG01

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

Material

The wild-type form of the human RhoA 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 RhoA-GST protein is 52 kDa. RhoA-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 5 mg/ml by the addition of 5 µl of distilled water. The protein will then be in the following buffer: 50 mM Tris pH 7.5, 100 mM NaCl, 1.0 mM $MgCl_2$, 5.0% sucrose and 1.0% 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 µg 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. RhoA-GST protein was determined to be >90% pure (see Figure 1). The minor contaminant at 28 kDa is the GST protein. This contaminant does not affect the activity of RhoA-GST.

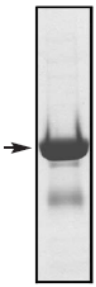

Figure 1. RhoA-GST Protein Purity Determination. A 20 µg sample of recombinant RhoA-GST protein (molecular weight approx. 52 kDa, arrow) was separated by electrophoresis in a 12% SDS-PAGE system and stained with Coomassie Blue. Protein quantitation was performed using the Precision Red Protein Assay Reagent (Cat. # ADV02).

Biological Activity Assay

The biological activity of RhoA-GST can be determined from its ability to catalyze the exchange of GDP for GTP. The human Dbs (DH/PH) protein is an exchange factor for RhoA and Cdc42, and is used with the RhoGEF exchange assay biochem kit (Cat. # BK100) to monitor the exchange ability of RhoA. The exchange rate (Vmax) of RhoA-GST is enhanced two fold in the presence of an equimolar amount of hDbs.

Reagents

1. Recombinant RhoA-GST protein (Cat. # RHG01)
2. Recombinant His-hDbs protein (Cat. # GE01)
3. 2x Exchange buffer (40 mM Tris pH 7.5, 300 mM NaCl, 20 mM $MgCl_2$, 2 mM DTT, 10%(w/v) sucrose, 2% (w/v) dextran, 100 µg/ml BSA, and 1.5 µM mant-GTP).

   All of the above reagents are available in the RhoGEF exchange assay biochem kit (Cat # BK100).

Equipment

1. Fluorescence spectrometer. Program the fluorimeter at an excitation filter wavelength of 360 nm and emission filter wavelength of 440 nm. The bandwidth of the filter should be no more than 20 nm or significant background noise and reduced sensitivity of the assay may be experienced. The fluorimeter should be at 20°C and set on kinetic mode, it is recommended to take a reading once every 30 seconds for at least 60 cycles. We recommend a TECAN SpectroFluro plus (GmbH, Austria) or Perkin-Elmer LS spectrometer.
2. Corning 96-well half area plates (Cat. # 3686) or other plate with low protein binding surface.

Method

1. 1) Resuspend the His-hDbs protein (Cat. # GE01) to 50 µM stock with the addition of 25 µl Milli-Q water. Keep on ice.
2. 2)Resuspend the RhoA-GST protein to give a 200 µM stock solution. Dilute an aliquot to 50 µM with Milli-Q water. Keep on ice.
3. 3) Add the following components together into four wells of a 96 well half area plate. Two wells will be the control reactions, and the others the test samples. Mix the components by gentle pipeting.

Volume per tube	Reagent
75 µl	2x Exchange buffer
3 µl	50 µM RhoA-GST
69 µl	Milli-Q water

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4. Insert the plate into the fluorimeter and begin reading.
5. After 5-10 cycles (150-300 seconds, you can set this time as time zero), add 3 µl of the His-hDbsprotein to the test wells and 3 µl of Milli-Q water to the control wells. Quickly mix the solutions by swirling with the tip or use the automix function where available. It is important to keep this mixing step as short as possible to obtain a smooth curve. Resume reading for at least 30 minutes.
6. The exchange rate can be calculated by reducing the data to Vmax with software that accompanies the plate reader. The exchange curve can be generated by exporting the raw data to Microsoft Excel.

Product Uses

- Study of RhoA binding proteins e.g. effectors, GAPs and GEFs.
- Identification of RhoA interacting proteins.
- Positive control for Western blots.
- Drug screening reagent (protein:protein inhibitors).

Product Citations/Related Products

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