V. 1.2

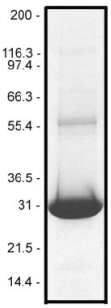
His-RhoA Protein: wild-type
(Human recombinant)
Cat. # RH01
Lot: 185 Amount: 1 x 100 µg
Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

Material
The wild-type human RhoA protein has been produced in a bacterial expression system. The recombinant protein contains six histidine residues at its amino terminus (His-tag). The molecular weight of His-RhoA is approximately 28 kDa. His-RhoA protein is supplied as a white lyophilized powder.

Storage and Reconstitution
Before reconstitution, briefly centrifuge to collect the product at the bottom of the tube. The protein should be reconstituted to 5 mg/ml with Milli-Q water (20 µl water per 100 µg protein). When reconstituted, the protein will be in the following buffer: 50 mM Tris pH 7.6, 50 mM NaCl, 0.5 mM $MgCl_2$, 5% (w/v) sucrose and 1% (w/v) 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 six months if stored at -70°C. The protein must not be exposed to repeated freeze-thaw cycles. The lyophilized protein is stable at 4°C desiccated (<10% humidity) for one year.

Purity
Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 4-20% polyacrylamide gradient gel. His-RhoA protein was determined to be 85% pure. (see Figure 1).

Figure 1. His-RhoA Protein Purity Determination. A 20 µg sample of recombinant His-RhoA protein (molecular weight approx. 28 kDa) was separated by electrophoresis in a 4-20% SDS-PAGE system and 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
The biological activity of His-RhoA 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 His-RhoA. Stringent quality control ensures that the exchange rate (Vmax) of His-RhoA is enhanced two fold in the presence of an equimolar amount of hDbs.

Reagents
1. Recombinant His-RhoA protein (Cat. # RH01)
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. Resuspend the His-hDbs protein (Cat. # GE01) to 2 mg/ml (50 µM) with the addition of 25 µl Milli-Q water. Keep on ice.
2. Resuspend the His-RhoA protein as described in the reconstitution section for a 200 µM solution. Dilute an aliquot to 50 µM with Milli-Q water. Keep on ice.
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 pipetting.

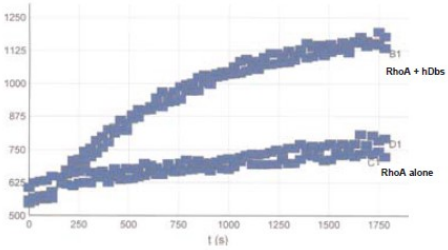
Volume per well	Reagent
75 µl	2x Exchange buffer
3 µl	50 µM His-RhoA
69 µl	Milli-Q water

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-hDbs protein (50 µM) 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.
7. A typical exchange curve is shown in Figure 2.

Figure 2. His-RhoA exchange assay.
His-RhoA protein (1 µM) was mixed with exchange buffer and aliquoted to two wells of a 96-well half area plate. After 5 cycles of reading in a fluorimeter, His-hDbs protein (1 µM) or Milli-Q water were added to the wells and the reactions were monitored for 30 min as described in the method.



Product Uses

- Study of RhoA interacting proteins, Effectors, GAPs and GEFs.
- Identification of RhoA interacting proteins.
- Positive control for Western blots.

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