

**His-RhoC Protein: Wild-type
(human recombinant)**

Cat. # RH03

Upon arrival store at 4°C (desiccated)

See datasheet for storage after reconstitution

Material

The wild-type human RhoC protein has been produced in a bacterial expression system. The recombinant protein contains six histidine residues (His-tag) at its amino terminus. The molecular weight of His-RhoC is approximately 30 kDa. His-RhoC 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 the addition of 20 µl of Milli-Q water. When reconstituted, the protein will be in the following buffer: 20 mM Tris pH 7.6, 0.5 mM MgCl₂, 0.5% (w/v) sucrose and 0.1% (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-RhoC protein was determined to be 90% pure. (see Figure 1).

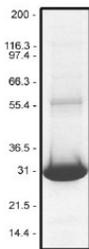


Figure 1. His-RhoC Protein Purity Determination. A 20 µg sample of recombinant His-RhoC protein (molecular weight approx. 30 kDa) was separated by electrophoresis in a 4-20% SDS-PAGE system and stained with Coomassie Blue. Protein quantitation was determined using the Precision Red Protein Assay Reagent (Cat. # ADV02). ?Mark12 molecular weight markers are from Invitrogen.

Biological Activity Assay

The biological activity of His-RhoC can be determined from its ability to catalyze the exchange of GDP for GTP. EDTA is used to create a conformational change in the His-RhoC protein that mimics GEF (Guanine nucleotide Exchange Factor) activity. The RhoGEF exchange assay biochem kit (Cat. # BK100) is used to monitor the exchange ability of His-RhoC. Stringent quality control ensures that the exchange rate (V_{max}) of His-RhoC is enhanced two fold in the presence of 40 mM EDTA.

Reagents

1. Recombinant His-RhoC protein (Cat. # RH03)
2. 400 mM EDTA loading buffer
3. 2x Exchange buffer (40 mM Tris pH 7.5, 300 mM NaCl, 20 mM MgCl₂, 2 mM DTT, 10% (w/v) sucrose, 2% (w/v) dextran, 100 µg/ml BSA, 1.5 µM mant-GTP).

2x Exchange buffer is 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 you may experience significant background noise and reduced sensitivity of the assay. 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 Spectro-Fluoro 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-RhoC protein as described in the reconstitution section to give a 200 µM solution. Dilute an aliquot to 50 µM with Milli-Q water. Keep on ice.
2. Add the following components together into four wells of a 96 well plate. Two wells will be the control reaction, and the others the test sample with EDTA. Mix the components by gentle pipeting.

<u>Volume per well</u>	<u>Reagent</u>
50 µl	2x Exchange buffer
4 µl	50 µM His-RhoC
36 µl	Milli-Q Water

3. Insert the plate into the fluorimeter and begin reading.
4. After 5-10 cycles (150-300 seconds, you can set this time as time zero), add 10 μ l of 400 mM EDTA to the test wells (40 mM final) and 10 μ l 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 min.
5. 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.
6. A typical exchange curve is shown in Figure 2.

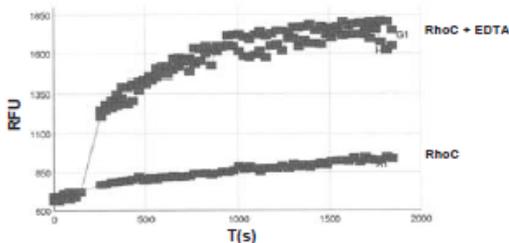


Figure 2. His-RhoC exchange assay. His-RhoC protein (1 μ M) was mixed with exchange buffer and aliquoted to four wells of a 96-well half area plate. After 5 cycles of reading in a fluorimeter, EDTA to 40 mM or Milli-Q water were added to the wells and the reactions monitored for 30 min as described in the method.

Product Uses

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

Product Citations/Related Products

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