V. 1.0

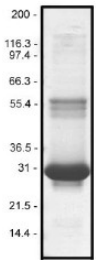
His-Ras Protein: wild-type
(Human recombinant)
Cat. # RS01

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

Material
The wild-type human Ras 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-Ras is approximately 28 kDa. His-Ras 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: 2 mM Tris pH 7.6, 0.5 mM $MgCl_2$, 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 a -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-Ras protein was determined to be 80% pure.



(see Figure 1).

Figure 1. His-Ras Protein Purity Determination. A 10 µg sample of recombinant His-Ras 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-Ras 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-Ras 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-Ras. Stringent quality control ensures that the exchange rate (Vmax) of His-Ras is enhanced two fold in the presence of 40 mM EDTA.

Reagents
1. Recombinant His-Ras protein (Cat. # RS01)
2. 400 mM EDTA loading buffer
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, 1.5 µM mant-GTP).

His-Ras and 2x Exchange buffer 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 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-Fluro 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-Ras 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 two will be test samples containing EDTA. Mix the components by gentle pipeting.

Volume per well	Reagent
50 µl	2 x Exchange buffer
4 µl	50 µM His-Ras
36 µl	Milli-Q Water

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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 µl of 400 mM EDTA to the test wells (40 mM final) and 10 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.
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.

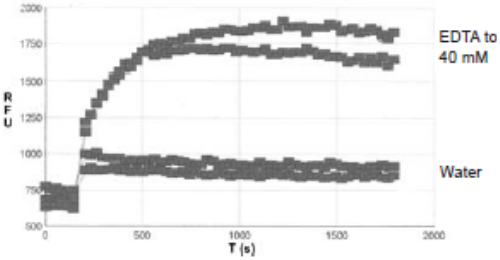

Figure 2. His-Ras exchange assay. His-Ras protein (1 µM) was mixed with exchange buffer and aliquoted to four wells of a 96-well half area plate. After 5 cycles of reading in a fluorimeter, EDTA was added to 40 mM in the test samples and Milli-Q water to the control samples. Reactions were monitored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

Product Uses

- Study of Ras exchange activity with different GEFs.
- Identification of Ras exchange factors (GEFs)
- Positive control for GEF studies.
- Biochemical characterization of Ras protein interactions
- Western blot standard

Product Citations/Related Products

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