V. 1.0

His-Rap1B protein
(Human recombinant)
Cat. # RR02

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

Material

The human Rap1B protein (Genbank accession NM_015686) has been expressed in E. coli. The recombinant protein contains six histidine residues (His-tag) at its amino terminus and has an approximate molecular weight of 25 kDa. Rap1B is a small GTPase of the Rap family of proteins which share similarity to the Ras superfamily (1). Rap signaling has been shown to be important in the regulation of multiple cellular processes including cell differentiation and adhesion (2 and references therein). His-Rap1B 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: 50 mM Tris pH 7.5, 50 mM NaCl, 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-Rap1B protein was determined to be 88% pure. (see Figure 1).

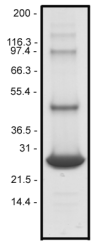

Figure 1. His-Rap1B Protein Purity Determination. A 20 µg sample of recombinant His-Rap1B protein (approx. 25 kDa) was separated by electrophoresis in a 4-20% SDS-PAGE system and stained with Coomassie Blue. Protein quantitation was determined using the Precision Red Protein Assay Reagent (Cat. # ADV02). Mark12 molecular weight markers are from Invitrogen.

Biological Activity Assay

The biological activity of His-Rap1B can be determined from its ability to catalyze the exchange of GDP for GTP. EDTA loading of a fluorescent analog of GTP (RhoGEF exchange assay biochem kit Cat. # BK100) is used to monitor the exchange ability of His-Rap1B in comparison to His-Ras. Stringent quality control ensures that the exchange rate (Vmax) of His-Rap1B is enhanced two fold in the presence of 40 mM EDTA.

Reagents

1. Recombinant His-Rap1B protein (Cat. # RR02)
2. Recombinant His-Ras protein (Cat. # RS01)
3. 400 mM EDTA loading buffer
4. 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 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-Rap1B 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.
2. Resuspend and dilute His-Ras protein to 50 µM with Milli-Q water. Keep on ice.
3. Add the following components together into eight wells of a 96 well plate. Two wells will be the Rap1B control reactions, two wells will be the Ras control reactions, and the remainder will be the test samples containing 40 mM EDTA. Mix the components by gentle pipeting.

Volume per well	Reagent
50 µl	2x Exchange buffer
4 µl	50 µM His-Rap1B or 50 µM His-Ras
36 µl	Milli-Q water

Phone: (303) 322.2254 **Fax:** (303) 322.2257
Customer Service: cserve@cytoskeleton.com
Technical Support: tservice@cytoskeleton.com

4. Insert the plate into the fluorimeter and begin reading.
5. After 5-10 cycles (150-300 seconds, 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 60 min.
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 curves comparing recombinant Rap1B and Ras is shown in Figure 2.

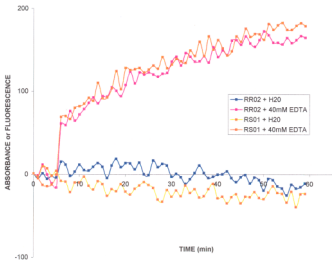

Figure 2. His-Rap1B and His-Ras Exchange Assay. His-Rap1B and His-Ras protein (1 µM) were mixed with exchange buffer and aliquoted to eight separate wells of a 96-well half area plate. After 5 cycles of reading in a fluorimeter, EDTA to 40 mM or Milli-Q water were added to the wells and the reactions monitored for 60 min as described in the method. An average exchange curve for each reaction was obtained in Microsoft Excel

References

1. Pizon, V., et al. *Nucleic Acids Res.* 1988. 16 (15), 7719.
2. Stork, P.J. *Trends Boichem Sci.* 2003 28:267-275.

Product Uses

- Study of Rap1B exchange activity with different GEFs.
- Identification of Rap1B exchange factors (GEFs)
- Positive control for other GEF studies.

Product Citations/Related Products

For the latest citations and related products please visit www.cytoskeleton.com.