V. 1.0

His-Rac1 Protein Wild-type
(Human recombinant)
Cat. # RC01

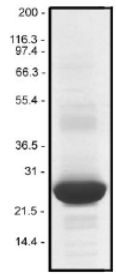
Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

Material
Wild-type human Rac1 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 the His-Rac1 is approximately 22 kDa. His-Rac1 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 Milli-Q water. When reconstituted, the protein will be in the following buffer: 50 mM Tris pH 7.5, 0.5 mM $MgCl_2$, 50 mM NaCl, 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 six months.

Purity
Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 12% polyacrylamide gradient gel. His-Rac1 protein was determined to be >90% pure. (see Figure 1).

Figure 1. His-Rac1 Protein Purity Determination. A 20 µg sample of recombinant His-Rac1 protein (molecular weight approx. 22 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-Rac1 can be determined from its ability to catalyze the hydrolysis of GTP (GTPase activity). The RhoGAP Assay Biochem Kit (Cat. # BK105) is used to monitor GTP hydrolysis by His-Rac1 in the presence of p50 RhoGAP. Stringent quality control ensures that the hydrolysis of GTP by His-Rac1 is enhanced three fold in the presence of the p50 RhoGAP.

Reagents
1. Recombinant His-Rac1 protein (Cat. # RC01)
2. RhoGAP Assay Biochem Kit (Cat. # BK105)

Equipment
1. Microplate spectrometer capable of reading at 650 nM. Cytoskeleton Inc. recommends the SpectroMax250 from Molecular Devices Inc.
2. Corning 96-well half area plate (Cat. # 3696) or other plate with low protein binding surface.

Method
1. Resuspend the His-Rac1 protein as described in the reconstitution section for a 250 µM solution. Dilute one aliquot to 50 µM with cold Milli-Q water. Keep on ice.
2. Prepare two reaction mixes according to the RhoGAP Assay Biochem Kit. Reaction mix #1 contains p50 RhoGAP (32 µg/100 µl) and will be used to monitor p50 RhoGAP activated GTPase activity. Reaction mix #2 contain no GAP protein and will be used to monitor intrinsic GTPase activity.
3. Prepare and dilute a GTP stock solution to 800 µM in cold Milli-Q water. Keep on ice.
4. Add the following proteins into duplicate wells of a 96 well plate on ice:
   Wells A1 and A2: 5 µl of 50 µM His-Rac1
   Wells B1 and B2: 5 µl of 50 µM His RhoA
   Wells C1 and C2: 5 µl of 50 µM His-Rac1
   Wells D1 and D2: 5 µl of 50 µM His RhoA
5. Pipette 25 µl of reaction reaction mix #1 into wells A1, A1, B1 and B2.
6. Pipette 25 µl of reaction reaction mix #2 into wells C1, C2, D1 and D2.
7. Using a multichannel pipette, add 10 µl of 800 µM GTP to each well and incubate at 37°C for 20 min. Shake the plate for 5 s to ensure complete solution mixing.
8. After 20 min, remove the plate and add 120 µl of Cytophos reagent (included in BK105) to each well and incubate at room temperature for 10 min.
9. Read the absorbance at 650 nm. A typical GAP assay result is shown in Figure 2.

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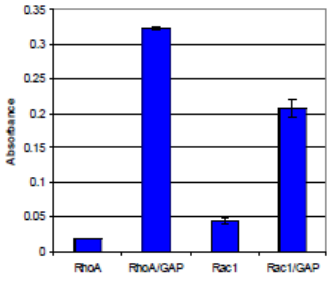

Figure 2. GTPase Activity of Recombinant Rac1 and RhoA.
Recombinant His-Rac1 and His-RhoA were assayed for GTPase activity using the RhoGAP Assay Biochem Kit (Cat. # BK105) as described. Each reaction contains +/- 5 µg His-Rac1, +/- 5 µg His -RhoA, +/- 8 µg p50 RhoGAP and 200 µM GTP. Reactions were incubated at 37°C for 20 min followed by the addition of Cytophos reagent for 10 min to determine the phosphate generated by the hydrolysis of GTP.
His-Rac1 shows a three fold increase in GTP hydrolysis in the presence of p50 RhoGAP. His-RhoA activity is shown for comparison.

Product Uses

- Control for the measurement of the GTP/GDP ratio of Rac1 In vitro.
- Identification of Rac1 binding proteins.
- Study of Rac1 function In vivo by the introduction of His-Rac1 into live cells
- Quantitation standard for activated Rac1 in tissue culture lysates

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