V. 2.0

His-Rac1 L61 Mutant Protein
Constitutively Active
Cat. # R6101

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

Material
The constitutively active form of the human Rac1 protein has been produced in a bacterial expression system. The protein has a glutamine to leucine substitution at amino acid 61, creating a constitutively active mutant protein that will not hydrolyze GTP. The recombinant protein contains six histidine residues (His-tag) at its amino terminus. The approximate molecular weight of His-L61 Rac1 protein is 28 kDa. His-Rac1 L61 protein is supplied as a white lyophilized powder.

Storage and Reconstitution
Before reconstitution, briefly centrifuge to collect the product to the bottom of the tube. The protein should be reconstituted to 1 mg/ml by the addition of 10 µl of distilled water. When reconstituted, the protein will be in the following buffer: 10 mM Tris pH 7.5, 10 mM NaCl, 0.1 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 at -70°C. The protein is stable for six months under these conditions. 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 gel. His-Rac1 L61 protein was determined to be 90% pure (see Figure 1). The higher molecular weight band in Fig. 1 is a doublet of R6101.

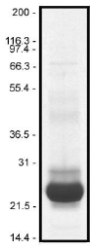

Figure 1. His-Rac1 L61 Protein Purity Determination. A 10 µg sample of recombinant His-Rac1 L61 protein (molecular weight approx. 28 kDa) was separated by electrophoresis in a 12% SDS-PAGE system, and stained with Coomassie Blue. Protein quantitation was performed using the Precision Red Protein Assay Reagent (Cat. # ADV02). Mark12 molecular weight markers are from Invitrogen.

Biological Activity Assay
His-Rac1 L61 mutant protein can bind GTP but its intrinsic GTPase activity has been eliminated, resulting in a constitutively active protein. A standard biological assay for His-Rac1 L61 activity consists of a pull-down assay using PAK-PBD beads (Cat. # PAK02). The PAK (p21 Activated Kinase CRIB domain) protein is an effector of Rac1, and will specifically bind to active GTP bound Rac1. Stringent quality control ensures that >80% of His-Rac1 L61 protein can be pulled down using this method.

Reagents
1. Recombinant His-Rac1 L61 constitutively active protein (Cat. # R6101)
2. Recombinant His-Rac1 wild-type protein (Cat. # RC01)
3. PAK-PBD beads (Cat. # PAK02)
4. Cell lysis buffer (50 mM Tris pH 7.5, 10 mM $MgCl_2$, 0.3 M NaCl, 2% IGEPAL)
5. Loading buffer (150 mM EDTA)
6. Stop buffer (600 mM $MgCl_2$)
7. Wash buffer (25 mM Tris pH 7.5, 30 mM $MgCl_2$, 40 mM NaCl)
8. GTPγS (20 mM solution) (Cat. # BS01)
9. GDP (100 mM solution)
10. BSA (10 mg/ml)
11. Anti-Rac1 monoclonal antibody (Cat. # ARC03)

Equipment
1. Microfuge at 4°C
2. SDS-PAGE and Western blot apparatus

Method
1. Dilute His-Rac1 L61 constitutively active protein to 0.1 µg/µl with cold cell lysis buffer.
2. Dilute His-Rac1 wild-type protein to 0.1 µg/µl with cold cell lysis buffer.
3. Resuspend PAK-PBD beads to 1 µg/µl by the addition of 500 µl cold distilled water.
4. Add 23 µl of cell lysis buffer and 2 µl of loading buffer into two microfuge tubes on ice.
5. Add 2 µl (200 ng) of His-Rac1 wild-type protein into both tubes.
6. Add 3 µl of GTPγS to one tube and 3 µl of GDP to the other tube. Incubate the loading reactions at room temperature for 15 min.
7. Repeat the nucleotide loading steps 4 through 6 with His-Rac1 L61 constitutively active protein.
8. Stop all reactions with the addition of 4 µl of stop buffer and place on ice.
9. Add 215 µl of cold cell lysis buffer and 20 µl of BSA to each

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tube on ice.

10. Add 10 µl (10 µg) of PAK-PBD beads to each tube and rotate for 30 min at 4°C
11. Pellet the beads at 6800 x g in a microfuge at 4°C for 1 min.
12. Remove the supernatant and wash the beads in 500 µl of wash buffer.
13. Pellet the beads as before and resuspend in 20 µl of SDS sample buffer.
14. The bead and supernatant samples can now be analyzed by Western blot using a Rac1 specific monoclonal antibody (Cat. # ARC03).
15. Typical assay results are shown in Figure 2.

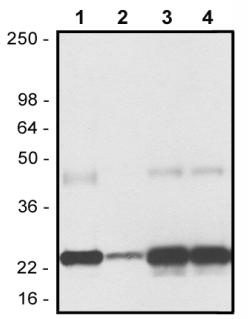

Figure 2. Binding of Wild-type and Constitutively active His-Rac1 to PAK-PBD beads *in vitro*. 200 ng of wild-type and constitutively active His-Rac1 protein were loaded with either GTPγS (lanes 1 and 3) or GDP (lanes 2 and 4), subjected to a pull-down assay with 10 µg of PAK-PBD beads and analyzed by Western blot using a Rac1 specific monoclonal antibody as described in the method. Lanes 1 and 2, wild-type His-Rac1. Lanes 3 and 4, constitutively active His-Rac1. Note: GTPγS and GDP samples look identical in the constitutively active His-Rac1 pull-down due to extremely poor nucleotide exchange. See Blue molecular weight markers are from Invitrogen.

Product Uses

- Identification of Rac1 binding proteins
- Study of Rac1 function *in vivo* by the introduction of constitutively active His-Rac1 into live cells

Product Citations/Related Products

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