Rac1-GST Protein
Wild-type
Cat. # RCG01
Lot # 324 Amount: 8 x 25 μg
Store at 4ºC (desiccated) or at -70ºC

Material

The wild-type form of the human Rac1 protein has been produced in a bacterial expression system. The recombinant protein is tagged with GST (28 kDa) at its amino terminus. The approximate molecular weight of the Rac1-GST protein is 45 kDa. Rac1-GST is supplied as a white lyophilized powder.

Storage and Reconstitution

Briefly centrifuge to collect the product at the bottom of the tube. The protein should be reconstituted to 5 mg/ml by the addition of 5 μl of distilled water. The protein will then be in the following buffer: 50 mM Tris pH 7.5, 100 mM NaCl, 1.0 mM $MgCl_2$, 5.0% sucrose and 1.0% 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 (10 μg is recommended for one assay), snap frozen in liquid nitrogen and stored at -70ºC. The protein is stable for 6 months if stored at -70ºC. The protein should not be exposed to repeated freeze-thaw cycles. The lyophilized protein is stable for 1 year if stored desiccated to <10% humidity at 4ºC.

Purity

Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 12% polyacrylamide gel. Rac1-GST protein was determined to be >90% pure (see Figure 1). The minor contaminant at 28 kDa is the GST protein. This contaminant does not affect the activity of Rac1-GST.

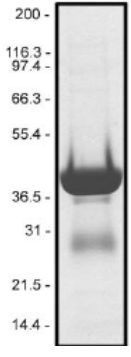

Figure 1. ***Rac1-GST Protein Purity Determination.*** A 20 μg sample of recombinant Rac1-GST protein (molecular weight approx. 45 kDa) was separated by electrophoresis in a 12% SDS-PAGE system and stained with Coomassie Blue. Protein quantitation was performed using the Precision Red Protein Assay Reagent (Cat. # ADV02). Mark12 molecular weight markers are from Invitrogen.

Biological Activity Assay

The biological activity of Rac1-GST can be determined from its ability to directly interact in its GTP bound form with effector proteins such as the PAK (p21 activated kinase) family of serine/threonine kinases (1). A standard biological assay for Rac1-GST consists of a PAK kinase pulldown from bovine brain extracts using Glutathione Sepharose beads.

Reagents

1) Recombinant wild-type Rac1-GST protein (Cat. # RCG01)
2) Recombinant dominant negative Rac1 N17-GST protein (Cat. # R17G01)
3) Recombinant constitutively active Rac1 L61-GST protein (Cat. # R61G01)
4) Bovine brain extract (20 mg/ml) prepared in 50 mM PIPES pH 7.0,130 mM NaCl, 1 mM PMSF, 1 mM DTT, 5 μg/ml leupeptin and 0.5% Triton X-100
5) Glutathione Sepharose™ 4B (Amersham Biosciences, Cat. # 27-4574-01)
6) Cell lysis buffer (50 mM Tris pH 7.5, 10 mM $MgCl_2$, 0.3 M NaCl and 2% IGEPAL)
7) Wash buffer (25 mM Tris pH 7.5, 30 mM $MgCl_2$ and 40 mM NaCl)
8) Loading buffer (150 mM EDTA)
9) Stop buffer (600 mM $MgCl_2$)
10) GTPγS (20 mM solution)
11) GDP (100 mM solution)
12) Protease inhibitor cocktail (100x) (Cat. # PIC02)
13) 10 mg/ml BSA (bovine serum albumin)
14) Anti-PAK polyclonal antibody (Santa Cruz, Cat. # SC-882)

Equipment

1) Microfuge at 4ºC
2) SDS-PAGE and Western blot apparatus

Method

1) Dilute the bovine brain extract to 1 mg/ml in cell lysis buffer containing 1x protease inhibitors.
2) Centrifuge the extract at 16k x g for 15 min at 4ºC to remove cell membranes and insoluble material. Keep on ice.
3) Dilute the wild-type, constitutively active and dominant negative forms of Rac1-GST to 1.0 mg/ml with cell lysis buffer. Keep all proteins on ice.
4) Place six microfuge tubes on ice and add 500 μl of cell lysis buffer to each tube.
5) Add 10 μg of wild-type Rac1-GST (Cat. # RCG01) into two tubes labeled 1 and 2. Keep both tubes on ice.
5) Add 10 μg of constitutively active Rac1-GST (Cat. # R61G01) into two tubes labeled 3 and 4. Keep both tubes on ice.
6) Add 10 μg of dominant negative Rac1-GST (Cat. # R17G01) into two tubes labeled 5 and 6. Keep both tubes on ice.
7) Add 50 μl of loading buffer to each of the six experimental tubes (final conc. 15 mM EDTA) .
8) Add 6 μl of GTPγS to tubes 1, 3 and 5 (final conc. 1.0 mM GTPγS).
9) Add 6 μl of GDP to tubes 2, 4 and 6 (final conc. 0.2 mM GDP).
10) Gently mix all the tubes and incubate at 37ºC for 30 min.
11) Add 60 μl of stop buffer to each tube (final conc. 60 mM $MgCl_2$), and place on ice.

12) Add 20 µl of 10 mg/ml BSA to each tube.
13) Prepare a 50% slurry of Glutathione Sepharose beads in cell lysis buffer, and add 20 µl of the bead slurry to each of the tubes.
14) Gently rotate the tubes at 4ºC for 30 min.
15) Centrifuge the tubes at 8k rpm at 4ºC for 1 min to pellet the protein-bead complexes. Remove the supernatant being careful not to disturb the bead pellet. Wash the beads once with 500 µl of cell lysis buffer. Remove as much of the supernatant as possible without disturbing the beads.
16) Add 600 µl (600 µg) of the bovine brain extract to each of tubes and rotate for 30 min at 4ºC.
17) Centrifuge the tubes at 8k rpm at 4ºC for 1 min to pellet the protein-bead complexes.
18) Remove the supernatant and wash the beads in 500 µl of wash buffer. Repeat the wash once more. Take care not to disturb the bead pellet.
19) Remove the supernatant and and resuspend the protein-beads in 20 µl of SDS sample buffer.
20) The protein samples can now be analyzed by Western blot using a PAK polyclonal antibody.
21) Typical assay results are shown in Figure 2.

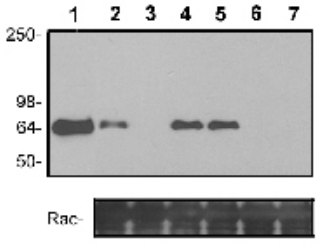

Figure 2. ***Recovery of PAK Kinases from Bovine Brain Extracts.*** Bovine brain extract (600 µg) was incubated with 10 µg of the following protein-bead complexes: Lane 1, 30 ug of bovine brain extract, lanes 2 and 3, wild-type Rac1, lanes 4 and 5, constitutively active Rac1, and lanes 6 and 7, dominant negative Rac1. Extracts in lanes 2, 4 and 6 were loaded with GTPγS whereas extracts in lanes 3, 5 and 7 were loaded with GDP. GST protein complexes were recovered by Glutathione Sepharose beads and subjected to Western blot analysis using a PAK polyclonal antibody.
Note: GTPγS and GDP samples look identical in the constitutively active Rac1 pull down due to extremely poor nucleotide exchange. Similarly, GTPγS and GDP samples look identical in the dominant negative Rac1 pull down due to poor nucleotide exchange. Separate Western blot shows the equal recovery of the various Rac1-GST complexes. SeeBlue molecular weight markers are from Invitrogen.

Product Uses

* Identification of active Rac1 binding proteins

References

1) Manser E., et al. 1994. *Nature*. 367:40-46.

Related Products

Cytoskeleton Inc. is the leading supplier of purified small G-proteins, visit our web site or call for information on the small G-proteins currently available. These include the small G-protein Activation Assay Kits, and a variety of affinity reagents for small G-protein activation assays:

* Rac Activation Assay Kit	Cat. # BK035
* Ras Activation Assay Kit	Cat. # BK008
* EasyRad Phosphate Assay Kit	Cat. # BK055
* RhoA Activation Assay Kit	Cat. # BK036
* RhoGEF Exchange Assay Kit	Cat. # BK100
* Rac1 GST protein: constitutively active	Cat. # R61G01
* Rac1 GST protein: dominant negative	Cat. # R17G01
* Rac1 GST beads: wild-type	Cat. # BR01
* Rac1 GST beads: constitutively active	Cat. # BR02
* Rac1 GST beads: dominant negative	Cat. # BR03
* Cdc42 GST protein: wild-type	Cat. # CDG01
* Cdc42 GST protein: dominant negative	Cat. # C17G01
* Cdc42 GST protein: constitutively active	Cat. # C61G01
* Cdc42 His protein: wild-type	Cat. # CD01
* H Ras GST protein: wild-type	Cat. # G21G01-C
* H Ras GST beads: wild-type	Cat. # BH01
* H Ras GST beads: constitutively active	Cat. # BH02
* RhoA GST protein: wild-type	Cat. # RHG02
* RhoA GST protein: constitutively active	Cat. # R63G01-A
* RhoA GST beads: wild-type	Cat. # BX01
* RhoA GST beads: constitutively active	Cat. # BX02
* Raf-RBD beads	Cat. # RF02