V. 1.0

PAK-GST Protein
(human p21 activated kinase PBD)
Cat. # PAK01

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

Material

The Rac/Cdc42 binding domain of the human p21 activated kinase 1 protein (PAK) has been overexpressed as a GST-tagged recombinant protein in a bacterial expression system. The recombinant protein (amino acids 67-150) includes the highly conserved PBD region (also referred to as the CRIB region) and sequences required for the high affinity interaction with GTP-Rac and GTP-Cdc42 proteins. The recombinant protein is tagged with GST (28 kDa) at its amino terminus and has an approximate molecular weight of 34 kDa. PAK-GST protein (250 µg protein per tube) 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 3 mg/ml by the addition of 83 µl of distilled water. When reconstituted, the protein will be in the following buffer: 50 mM Tris pH 7.5, 50 mM NaCl, 1.0% (w/v) dextran and 5% (w/v) sucrose. For storage, the reconstituted protein should be aliquoted into experiment sized amounts, snap frozen in liquid nitrogen and stored at -70°C. Under these conditions the protein is stable for 6 months. **To maintain high biological activity, the protein should not be exposed to repeated freeze thaw cycles.** The lyophilized protein is stable for 6 months if stored desiccated to <10% humidity at 4°C.

Purity

Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 12% SDS polyacrylamide gel. PAK-GST protein was determined to be 88% pure (see Figure 1).

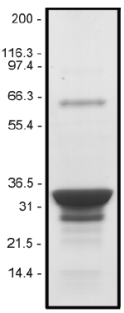

Figure 1. PAK-GST Protein Purity Determination. A 20 µg sample of PAK-GST protein (molecular weight approx. 34 kDa) was separated by electrophoresis in a 12% SDS-PAGE system and stained with Coomassie Blue. Protein quantitation was performed using the Precision Red Protein Assay Reagent (Cat. # ADV02). Mark12™ molecular weight markers are from Invitrogen.

Biological Activity Assay

PAK-GST protein specifically recognizes and binds the active "GTP-bound" forms of the Rac and Cdc42 proteins (1). It has a much lower affinity for the inactive "GDP-bound" forms of Rac and Cdc42. When coupled to a colored glutathione sepharose matrix, the PAK-GST protein beads become a convienent tool for assaying the activity of the Rac and Cdc42 proteins. A standard biological assay for PAK-GST protein consists of a Rac protein pulldown from human platelet extracts loaded with either GTPγS or GDP.

Reagents

1. PAK-GSTprotein (Cat. # PAK01)
2. Loading buffer (150 mM EDTA)
3. Stop buffer (600 mM $MgCl_2$)
4. Wash buffer (25 mM Tris pH 7.5, 30 mM $MgCl_2$, 40 mM NaCl)
5. Cell lysis buffer (50 mM Tris pH 7.5, 10 mM $MgCl_2$, 0.3 M NaCl, 2% IGEPAL)
6. GTPγS (20 mM solution)
7. GDP (100 mM solution)
8. Human platelet extract prepared in 50 mM PIPES pH 7.0,130 mM NaCl, 1 mM PMSF, 1 mM DTT, 5 µg/ml leupeptin, 0.5% Triton X-100
9. Protease inhibitor cocktail (Cat. # PIC02)
10. Anti-Rac polyclonal antibody (Cat. # ARC03)
11. Glutathione beads

Equipment

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

Method

1. Dilute the platelet extract to 1 mg/ml with cell lysis buffer containing protease inhibitors.
2. Centrifuge the extract at 14k rpm at 4°C for 5 min to pellet cell membranes and insoluble material.
3. Aliquot 500 µg (500 µl) of the extract into two experimental tubes.
4. Add 1/10th the volume of loading buffer to each tube (final conc. 15 mM EDTA).
5. Add 1/100th the volume of GDP to one tube (final conc. 1.0 mM GDP).
6. Add 1/100th the volume of GTPγS to the other tube (final conc. 0.2 mM GTPγS).
7. Incubate both tubes at room temperature for 15 min.
8. Stop the reaction by adding 1/10th the volume of stop buffer to each tube (final conc. 60 mM $MgCl_2$).
9. Resuspend PAK-GST protein to 3 mg/ml and add 20 µg (6.7 µl) protein to each reaction tube.

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10. Gently rotate the tubes at 4°C for 30 minutes.
11. Add 10 ul of glutathione beads (equilibrated in cell lysis buffer) to each reaction and incubate on the rotator for a further 30 minutes at 4°C.
12. Centrifuge the beads at 8k rpm at 4°C for 1 min.
13. Remove the supernatant and wash the beads in 500 µl of wash buffer.
14. Pellet the beads and resuspend in 20 µl of SDS sample buffer.
15. The protein samples can now be analyzed by Western blot procedure using a Rac1 monoclonal antibody (Cat. # ARC03). NOTE: The PAK01 can also be used in a Cdc42 activation assay using anti-Cdc42 monoclonal antibody (Cat. # ACD03).
16. Typical assay results are shown in Figure 2.

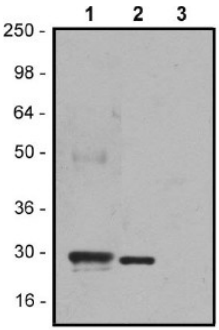

Figure 2. Selective Binding of PAK-GST Protein to the GTP-bound Form of Rac1 *In Vitro*. Human platelet extracts (500 µg) were loaded with either GTPγS (lane 2), or GDP (lane 3) as described in the method. The extracts were then incubated with 20 µg of PAK-GST protein, subsequently the PAK-GST was captured by addition of glutathione beads. The protein-bead complexes were recovered by centrifugation and subjected to Western blot analysis using a Rac1 specific polyclonal antibody. Lane 1 shows 50 ng of recombinant Rac-His control protein (Note: His-Rac1 runs slightly higher than endogenous Rac1 due to the presence of the 6x-His tag). SeeBlue™ molecular weight markers are from Invitrogen.

Product Uses

- Measurement of the GTP/GDP ratio of Rac or Cdc42 *in vitro*.
- Quantitation of GTP-Rac/Cdc42 from tissue and tissue culture cell lysates.

References

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

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