

Product Specification

PAK 7, active

(Full-length recombinant protein expressed in Sf 9 cells)

Catalog #: 7727
Lot #: _____
Aliquot size: 5 µg protein in 50 µl
Specific activity: 55 nmol/min/mg

Quality Control Analysis

Activity assessment

PAK7 protein (100 ng/µl concentration) was diluted to 50ng/µl with assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM MgCl₂, 0.05 mM DTT), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the Akt substrate peptide (CKRPRAASFAE) in the following assay condition:

- 10 µl diluted PAK7 protein
- 10 µl Akt substrate peptide (1 mg/ml stock)
- 5 µl [³²P] ATP mixture (250 µM ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [³²P] ATP, were incubated at 30° C and the reaction started by the addition of [³²P] ATP. After 15 minutes, the reaction was terminated by spotting 20 µl of the reaction mixture onto a phosphocellulose P81 paper. The P81 paper was dried and washed several times in 1% phosphoric acid prior to counting in the presence of scintillation fluid in a scintillation counter. The actual counts, using various dilution of PAK7 in the assay, are shown in Fig. 1.

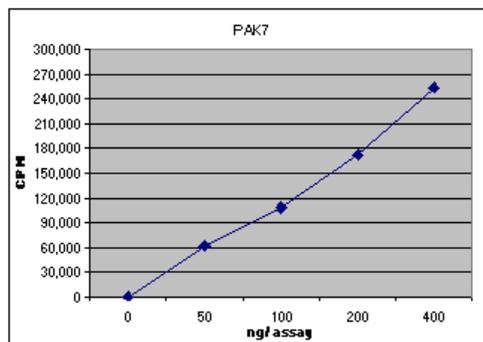


Fig. 1 PAK7 activity assay

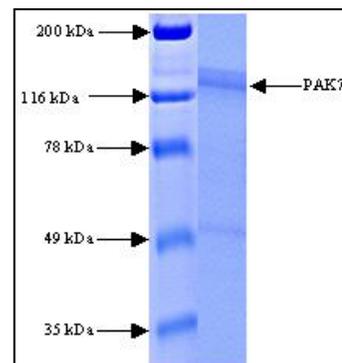


Fig. 2 PAK7 protein gel

Purity assessment

1 µg of PAK7 protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >75% purity of the PAK7 product, and the band was at ~130 kDa (Fig. 2).

Product Description

Recombinant full length human PAK7 containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells. The gene accession number is NM_177990.
This material is sold for research purposes only.

Specific Activity

55 nmol phosphate incorporated into the Akt substrate peptide (CKRPRAASFAE) per minute per mg protein at 30° C for 15 minutes using a final concentration of 50 µM ATP (0.83 µCi/assay).

Formulation

Recombinant protein in storage buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol).

Storage and Stability

Store product frozen at or below -70° C. Stable for 1 year at -70° C as undiluted stock. Aliquot to avoid repeated thawing and freezing.

Scientific Background

The p21-activated kinase (PAK) family of protein kinases has recently attracted considerable attention as an effector of Rho family of small G proteins and as an upstream regulator of MAPK signalling pathways during cellular events such as re-arrangement of the cytoskeleton and apoptosis. PAK7 is a novel human PAK family kinase that contains a CDC42/Rac1 interactive binding (CRIB) motif at the N-terminus and a Ste20-like kinase domain at the C-terminus. PAK7 like the other Paks has been implicated in the regulation of cell morphology, motility and transformation.