

## Product Specification

### **PAK 4, active**

(Full-length recombinant protein expressed in Sf9 cells)

Catalog # 7707-5

Lot # \_\_\_\_\_

Aliquot size: 5 µg protein in 50 µl

Specific activity: 83 nmol/min/mg

### **Quality Control Analysis**

#### Activity assessment

PAK4 protein (100 ng/µl concentration) was diluted to 20ng/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 MgCl<sub>2</sub>, 0.05 mM DTT), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the PKB-sub peptide (CKRPRAASFAE) in the following assay condition:

10 µl diluted PAK4 diluted protein

10 µl PKB-sub peptide (1 mg/ml stock)

5 µl [<sup>32</sup>P] ATP mixture (250 µM ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [<sup>32</sup>P] ATP, were incubated at 30°C and the reaction started by the addition of [<sup>32</sup>P] 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 PAK4 in the assay, are shown in Fig. 1.

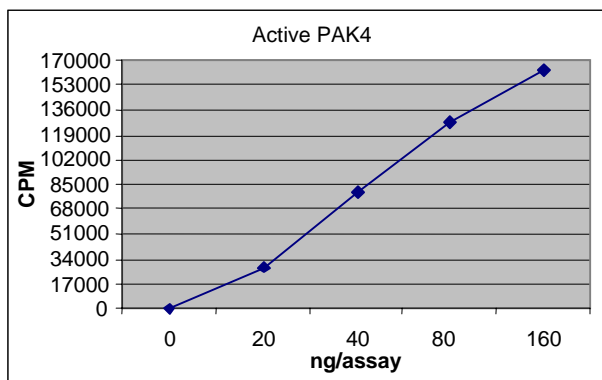


Fig. 1 PAK4 activity assay

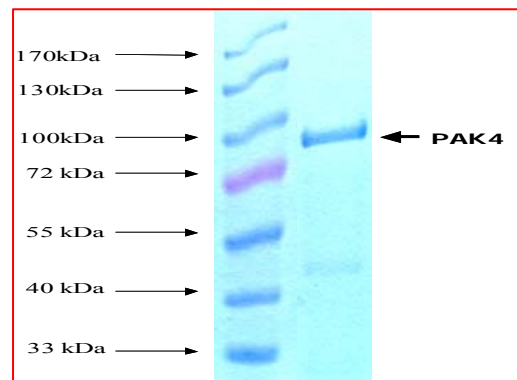


Fig. 2 PAK4 protein ge

### Purity assessment

1 µg of PAK4 protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >95% purity of the PAK4 product, and the band was at ~90 kDa (Fig. 2)

### **Product Description**

Recombinant full length human PAK4 containing N-terminal GST tag was expressed by baculovirus in Sf9 insect cells.

The gene accession number is NM\_005884.

This material is sold for research purposes only.

### Specific Activity

83 nmol phosphate incorporated into the PKB-sub peptide (CKRPRAASFAE) per minute per mg protein at 30<sup>0</sup>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<sup>0</sup>C. Stable for 1 year at -70<sup>0</sup>C as undiluted stock. Aliquot to avoid repeated thawing and freezing.

### Scientific Background

PAK4 is a recently identified member of the p21-activated kinases (PAKs) which have been implicated in the regulation of cell morphology, motility and transformation. These serine/threonine kinases are activated by and are effectors of small GTPases, cdc 42 and Rac. PAK4 belongs to the Group II PAKs which also includes PAK5 and PAK6 while Group I PAKs comprise of PAK1, PAK2 and PAK3. PAK4 differs from other members of the PAK family both in sequence and function. PAK4 has been shown to regulate cell morphology and cytoskeletal organization in mammalian cells. PAK4 regulates the activity of LIM kinase 1 which in turn phosphorylates Cofilin leading to cytoskeletal changes. PAK4 can protect cells from apoptosis in response to several different types of stimuli by inhibiting the pro-apoptotic proteins Bad and Caspase 8. PAK4 has been shown to associate with and mediate the downstream signaling of the keratinocyte growth factor receptor. In addition, PAK4 interacts with the β<sub>5</sub> integrins and regulates cell migration during engagement of the α<sub>v</sub>β<sub>5</sub> integrin receptor.