

## Certificate of Analysis

### PAK6, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-633, 14-633-K, 14-633M

Parent Lot # 1623030

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

**Product Description:** N-terminal 6His-tagged, recombinant, human PAK6, amino acids 382–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni<sup>2+</sup>-NTA agarose. Purity 94.6% by SDS-PAGE and Coomassie blue staining. MW = 37.9kDa.

**Specific Activity (Parent lot# 1623030):** 1961U/mg, where one unit of PAK6, active activity is defined as 1nmol phosphate incorporated into PAKtide per minute at 30°C with a final ATP concentration of 100µM.

**Formulation:** 1.88mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

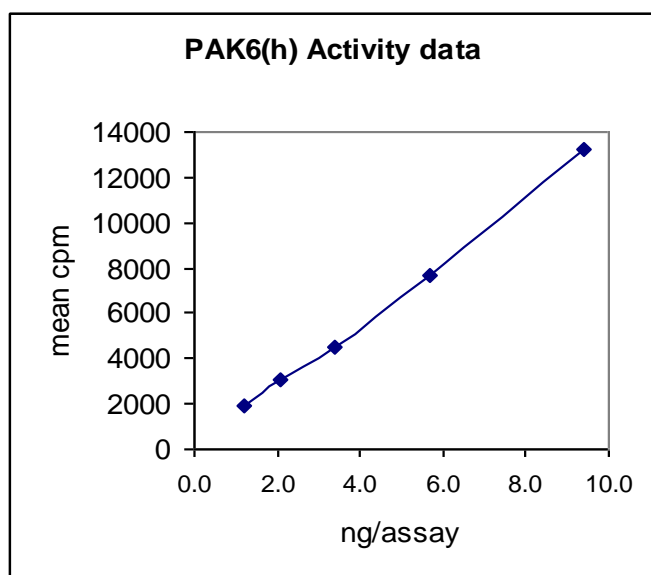
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**Handling Recommendations:** Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

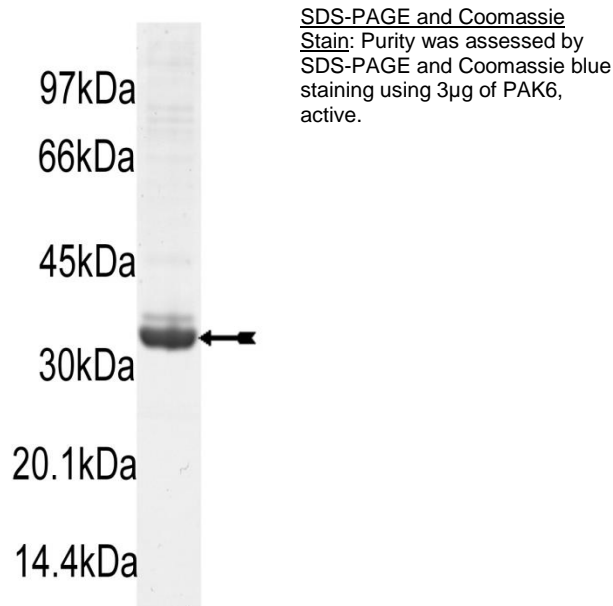
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 1.2–9.4ng of this lot of enzyme phosphorylated 200µM PAKtide in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



**MS Tryptic Fingerprint:** Confirmed product identity as PAK6 with the translated native sequence listed on page three.



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### Kinase Assay Protocol

#### Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **PAKtide (RRRLSFAEPG):** Use at a final assay concentration of 200 $\mu$ M. Prepare 2mM stock and add 2.5 $\mu$ l of stock per assay point.
3. **PAK6, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.2–9.4ng per assay point.
4. **[ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x magnesium acetate/[ $\gamma$ -<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [ $\gamma$ -<sup>33</sup>P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

#### Assay Procedure (96 well plate format) :

1. Add 5 $\mu$ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 $\mu$ l of **PAKtide**.
3. Add **2.5 $\mu$ l (1.2–9.4ng) PAK6, active**.
4. Add 5 $\mu$ l of dH<sub>2</sub>O.
5. Add 10 $\mu$ l of diluted [ $\gamma$ -<sup>33</sup>P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 $\mu$ l of 3% phosphoric acid.
8. Transfer a 10 $\mu$ l aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 $\mu$ l of 30% phosphoric acid.

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### PAK6 Sequence Information

<b><u>Protein</u></b>	human PAK6
<b><u>Tags</u></b>	N-terminal 6His
<b><u>Native sequence</u></b>	T31 of the recombinant protein is equivalent to T382 of native human PAK6
<b><u>Accession number</u></b>	GenBank NM_020168

#### ***Recombinant PAK6 amino acid sequence:***

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1 MSYYHHHHH DYDIPTTENL YFQGAMDPEF TGVVTHEQFK AALRMVVDQG DPRLLLDYSY
61 KIGEGSTGIV CLAREKHSGR QVAVKMDLR KQQRRELLFN EVVIMRDYQH FNVVEMYKSY
121 LVGEELWVLM EFLQGALTD IVSQVRLNEE QIATVCEAVL QALAYLHAQG VIHEDIKSDS
181 ILLTLDGRVK LSDFGFCAQI SKDVPKRKSL VGTPYWMAPE VISRSLYATE VDIWSLGIMV
241 IEMVDGEPY FSDSPVQAMK RLRDSPPPKL KNSHKVSPVL RDFLERMLVR DPQERATAQE
301 LLDHPFLLQT GLPECLVPLI QLYRKQTSTC

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#### ***Recombinant PAK6 nucleotide sequence:***

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc acagggtgtg tgacacatga gcagttcaag
121 gctgcgtca ggtgggtgtg ggaccagggt gacccccggc tgctgctgga cagctacgtg
181 aagattggcg agggctccac cggcatcgct tgcttggccc gggagaagca ctcgggccgc
241 cagggtggcg tcaagatgat ggacctcagg aagcagcagc gcaggagagc gctcttcaac
301 gaggtggatga tcatgcggga ctaccagcac ttcaacgtgg tggagatgta caagagctac
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421 atcgtctccc aagtcaggct gaatgaggag cagattgcca ctgtgtgtga ggctgtgctg
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661 gtgatctcca ggtctttgta tgccactgag gtggatatct ggtctctggg catcatgggtg
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961 cagctctacc gaaagcagac ctccacctgc tga

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