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Certificate of Analysis

PAK2, active

(Recombinant enzyme expressed in *E.coli* cells)
Item # 14-481, 14-481-K, 14-481M
Parent Lot # 1685379

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: *C*-terminal and *N*-terminal 6His-tagged and *N*-terminal T7 tagged, recombinant, human PAK2 amino acids 3–end, expressed in *E.coli* cells. Purified using Ni^{2+}/NTA-agarose. Purity 71.6% by SDS-PAGE and Coomassie blue staining. MW = 62.8kDa.

Specific Activity (Parent lot# 1685379): 3216U/mg, where one unit of PAK2, active activity is defined as 1nmol phosphate incorporated into 30µM Long S6 peptide per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 4.339mg/ml of enzyme in 50mM Tris/HCl pH 7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

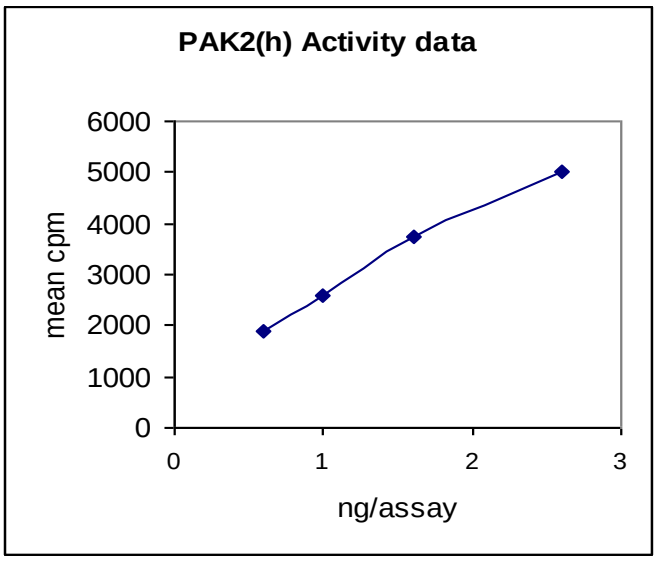
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

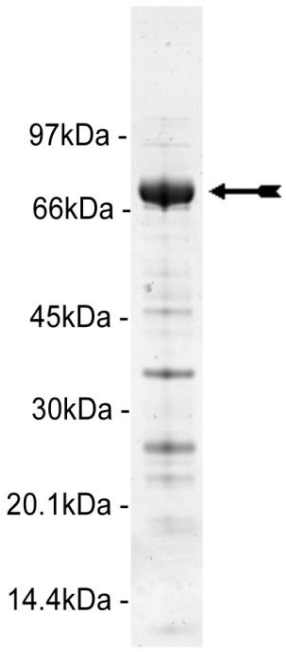
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.6–2.6ng of this lot of enzyme phosphorylated 30µM Long S6 peptide 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 identity as PAK2 with the translated sequence listed on page three



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of PAK2, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Long S6 substrate peptide:** Use at a final assay concentration of 30µM. Make up a 300µM stock. Add 2.5µl of stock per assay point.
3. **PAK2, active:** Dilute in 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.6–2.6ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5µl of reaction buffer per assay to wells.
2. Add 2.5µl of **Long S6 substrate peptide**.
3. Add **2.5µl (0.6–2.6ng) PAK2, active**.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl 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 1µl of 30% phosphoric acid.

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PAK2 Sequence Information

Protein Human PAK2

Tags *N*-terminal and *C*-terminal 6His-tagged and *N*-terminal T7 tagged

Native sequence D37 of the recombinant protein is equivalent to D3 of human PAK2

Accession number GenBank NM_U24153. The recombinant protein contains the amino acid substitutions L150F, P225T, V383I and ΔA339 with respect to this accession number. L150F, P225T and ΔA339 are reported in GenBank NM_U25975. The residue co ordinates in the native sequence have been given.

Recombinant PAK2 amino acid sequence:

```
   1 MGSSHHHHHH SSGLVPRGSH MASMTGGQQM GRGSMLDNGE LEDKPPAPPV RMSSTIFSTG
  61 GKDPLSANHS LKPLPSVPEE KKPRHKIISI FSGTEKGSKK KEKERPEISP PSDFEHTIHV
 121 GFDAVTGEFT GMPEQWARLL QTSNITKLEQ KKNPQAVLDV LKFYDSNTVK QKYLSFTPPE
 181 KDGFPSGTPA LNAKGTEAPA VVTEEEDDDE ETAPPVIAPR PDHTKSIYTR SVIDPVPAPV
 241 GDSHVDGAAK SLDKQKKKTK MTDEEIMEKL RTIVSIGDPK KKYTRYEKIG QGASGTVFTA
 301 TDVALGQEVA IKQINLQKQP KKELIINEIL VMKELKNPNI VNFLDSYLVG DELFVVMEYL
 361 AGGSLTDVVT ETCMDEAQIA AVCRECLQAL EFLHANQVIH RDIKSDNVLL GMEGSIKLTD
 421 FGFCAQITPE QSKRSTMVGT PYWMAPEVVT RKAYGPKVDI WSLGIMAIEM VEGEPPYLNE
 481 NPLRALYLIA TNGTPELQNP EKLSPIFRDF LNRCLEMDVE KRGSAKELLQ HPFLKLAKPL
 541 SSLTPLIMAA KEAMKSNRLE HHHHHH
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Recombinant PAK2 nucleotide sequence:

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   1 atgggcagca gccatcatca tcatcatcac agcagcggcc tggtgccgcg cggcagccat
  61 atggctagca tgactggtgg acagcaaatg ggtcgcggat ccatgctcga taacggagaa
 121 ctggaagata agcctccagc acctcctgtg cgaatgagca gcaccatctt tagcactgga
 181 ggcaaagacc ctttgtcagc caatcacagt ttgaaacctt tgccctctgt tccagaagag
 241 aaaaagccca ggcataaaat catctccata ttctcaggca cagagaaagg aagtaaaaag
 301 aaagaaaagg aacggccaga aatttctcct ccatctgatt ttgagcacac catccatgtt
 361 ggctttgatg ctgttactgg agaattcact ggcatgccag aacagtgggc tcgattacta
 421 cagacctcca atatcaccaa actagagcaa aagaagaatc ctcaggctgt gctggatgtc
 481 ctaaagttct acgactccaa cacagtgaag cagaaatatc tgagctttac tcctcctgag
 541 aaagatggct ttccttctgg aacaccagca ctgaatgcca agggaacaga agcacccgca
 601 gtagtgacag aggaggagga tgatgatgaa gagactgctc ctcccgttat tgccccgcga
 661 ccggatcata cgaaatcaat ttacacacgg tctgtaattg accctgttcc tgcaccagtt
 721 ggtgattcac atgttgatgg tgctgccaag tctttagaca aacagaaaaa gaagactaag
 781 atgacagatg aagagattat ggagaaatta agaactatcg tgagcatagg tgaccctaag
 841 aaaaaatata caagatatga aaaaattgga caaggggctt ctggtacagt tttcactgct
 901 actgacgttg cactgggaca ggaggttgct atcaaacaaa ttaatttaca gaaacagcca
 961 aagaaggaac tgatcattaa cgagattctg gtgatgaaag aattgaaaaa tcccaacatc
1021 gttaactttt tggacagtta cctggtagga gatgaattgt ttgtggtcat ggaatacctt
1081 gctggggggt cactcactga tgtggtaaca gaaacgtgca tggatgaagc acagattgct
1141 gctgtatgca gagagtgttt acaggcattg gagtttttac atgctaatca agtgatccac
1201 agagacatca aaagtgacaa tgtacttttg ggaatggaag gatctattaa gctcactgac
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1321 ccatactgga tggcaccaga ggtggttaca cggaaagctt atggccctaa agtcgacata
1381 tggtctctgg gtatcatggc tattgagatg gtagaaggag agcctccata cctcaatgaa
1441 aatcccttga gggccttgta cctaatagca actaatggaa ccccagaact tcagaatcca
1501 gagaaacttt ccccaatatt tcgggatttc ttaaatcgat gtttggaaat ggatgtggaa
1561 aaaaggggtt cagccaaaga attattacag catcctttcc tgaaactggc caaaccgtta
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1621 tctagcttga caccactgat catggcagct aaagaagcaa tgaagagtaa ccgtctcgag
1681 caccaccacc accaccactg a
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Reviewed and approved by site quality representative.