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Certificate of Analysis

PAK3, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-683, 14-683-K, 14-683M
Parent Lot # D13BP002N

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 6His-tagged, recombinant, human full length, PAK3 expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}-NTA agarose. Purity 75% by SDS-PAGE and Coomassie blue staining. MW = 63.2kDa.

Specific Activity (Parent lot# D13BP002N): 7976U/mg, where one unit of PAK3 activity is defined as 1nmol phosphate incorporated into PAKtide per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.817mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 20mM β-glycerophosphate, 10mM NaF, 0.25mM sodium orthovanadate, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

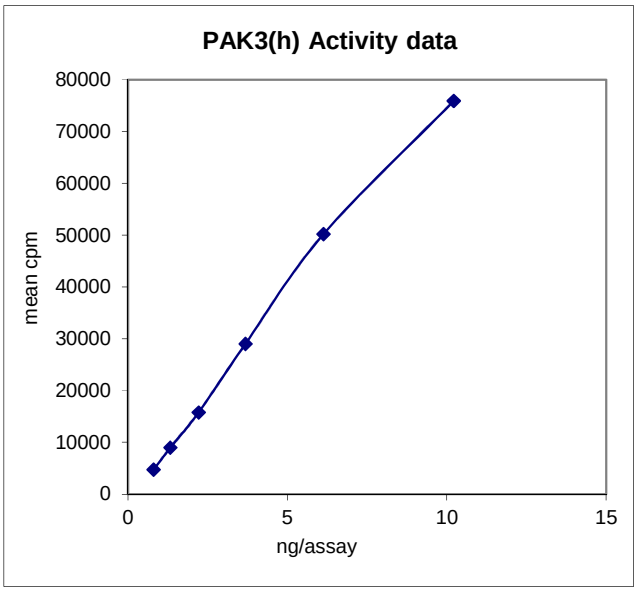
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

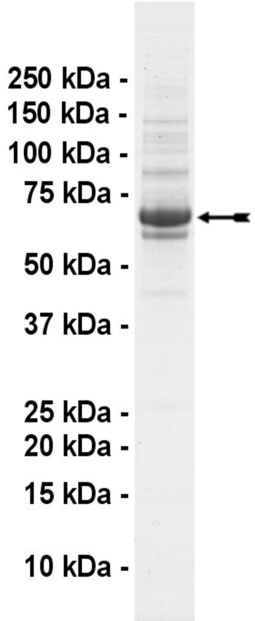
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 1–10ng 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 PAK3 with of the translated native sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of PAK3, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **PAKtide:** Use at a final concentration of 200µM. Make a 2mM stock. Add 2.5µl of stock per assay point.
3. **PAK3, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1–10ng 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 5 x reaction buffer per assay to wells.
2. Add 2.5µl of 200µM **PAKtide**.
3. Add **2.5µl (1–10ng) PAK3, 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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PAK3 Sequence Information

Protein Human PAK3

Tags *C*-terminal 6His

Native sequence M1 of recombinant protein is equivalent to M1 of native human PAK3

Accession number GenBank AB102659

Recombinant PAK3 amino acid sequence:

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  1 MSDGLDNEEK PPAPPLRMNS NNRDSSALNH SSKPLPMAPE EKNKKARLRS IFPGGGDKTN
 61 KKKEKERPEI SLPSDFEHTI HVGFDAVTGE FTPDLYGSQM CPGKLPEGIP EQWARLLQTS
121 NITKLEQKKN PQAVLDVLKF YDSKETVNNQ KYMSFTSGDK SAHGYIAAHP SSTKTASEPP
181 LAPPVSEEED EEEEEEEDEN EPPPVIAPRP EHTKSIYTRS VVESIASPAV PNKEVTPPSA
241 ENANSSTLYR NTDRQRKKSK MTDEEILEKL RSIVSVGDPK KKYTRFEKIG QGASGTVYTA
301 LDIATGQEVA IKQMNLQQQP KKELIINEIL VMRENKNPNI VNYLDSYLVG DELWVVMEYL
361 AGGSLTDVVT ETCMDEGQIA AVCRECLQAL DFLHSNQVIH RDIKSDNILL GMDGSVKLTD
421 FGFCAQITPE QSKRSTMVGT PYWMAPEVVT RKAYGPKVDI WSLGIMAIEM VEGEPPYLNE
481 NPLRALYLIA TNGTPELQNP ERLSAVFRDF LNRCLEMDVD RRGSAKELLQ HPFLKLAKPL
541 SSLTPLIIAA KEAIKNSSRH HHHHH
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Recombinant PAK3 nucleotide sequence:

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   1 atgtctgacg gtctggataa tgaagagaaa cccccggctc ctccactgag gatgaatagt
  61 aacaaccggg attcttcagc actcaaccac agctccaaac cacttcccat ggcccctgaa
 121 gagaagaata agaaagccag gcttcgctct atcttcccag gaggagggga taaaaccaat
 181 aagaagaagg agaaagagcg cccagagatc tctcttcctt cagactttga gcatacgatt
 241 catgtggggt ttgatgcagt caccggggaa ttcactccag atctctatgg ctcacagatg
 301 tgccccggga agctcccaga gggaattcca gagcaatggg cacgattact ccaaacttcc
 361 aacataacaa aattggaaca gaagaagaac ccacaagctg ttctagatgt tctcaaattc
 421 tatgattcca aagaaacagt caacaaccag aaatacatga gctttacatc aggagataaa
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 541 ttggcccctc ctgtgtctga agaagaagat gaagaggaag aagaagaaga agatgaaaat
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 721 gaaaatgcca attccagtac tttgtacagg aacacagatc ggcaaagaaa aaaatccaag
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 841 aaaaaataca caagatttga aaaaattggt caaggggcat caggtactgt ttatacagca
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1021 gttaattatt tagatagcta cttggtgggt gatgaactat gggtagtcat ggaatacttg
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1201 agagatataa agagtgacaa tattcttctc gggatggatg gctctgttaa attgactgac
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1321 ccatattgga tggcacctga ggtggtgact cgaaaagctt atggtccgaa agttgatatc
1381 tggtctcttg gaattatggc aattgaaatg gtggaaggtg aacccccttа ccttaatgaa
1441 aatccactca gggcattgta tctgatagcc actaatggaa ctccagagct ccagaatcct
1501 gagagactgt cagctgtatt ccgtgacttt ttaaatcgct gtcttgagat ggatgtggat
1561 aggcgaggat ctgccaagga gcttttgcag catccatttt taaaattagc caagcctctc
1621 tccagcctga ctcctctgat tatcgctgca aaggaagcaa ttaagaacag cagccgccat
1681 caccatcacc atcactaa
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