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Certificate of Analysis

PAK5, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-699, 14-699-K, 14-699M
Parent Lot # D8CN021U

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 PAK5, amino acids 425–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 95.8% by SDS-PAGE and Coomassie blue staining. MW = 37.4kDa.

Specific Activity (Parent lot# D8CN021U): 2565U/mg, where one unit of PAK5, active activity is defined as 1nmol phosphate incorporated into PAKtide per minute at 30°C with a final ATP concentration of 100µM.

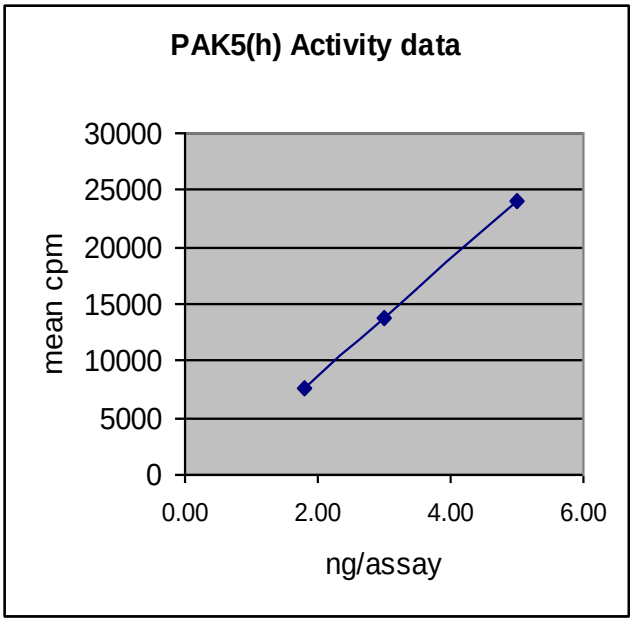
Formulation: 2.607mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date 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

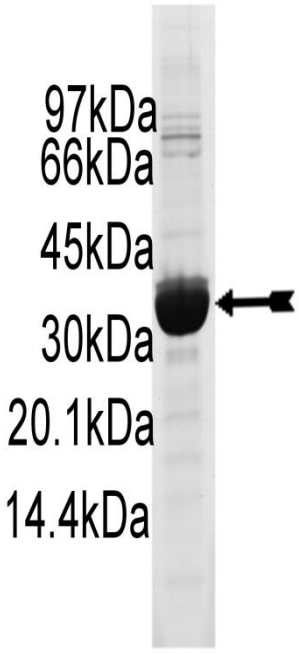
Quality Control Testing

Kinase Assay: 1.81–5.00ng 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 PAK5 with 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 PAK5, active.



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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 200µM. Prepare 2mM stock and add 2.5µl of stock per assay point.
3. **PAK5, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.81–5.00ng 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 **PAKtide**.
3. Add **2.5µl (1.81–5.00ng) PAK5, 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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PAK5 Sequence Information

Protein Human PAK5

Tags *N*-Terminal 6His

Native sequence GenBank NM_020341

Accession number S31 of recombinant sequence is equivalent to S425 of human PAK5

Recombinant PAK5 amino acid sequence:

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  1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF SRVSHEQFRA ALQLVVSPGD PREYLANFIK
 61 IGEGSTGIVC IATEKHTGKQ VAVKKMDLRK QQRRELLFNE VVIMRDYHHD NVVDMYSSYL
121 VGDELWVVME FLEGGALTDI VTHTRMNEEQ IATVCLSVLR ALSYLHNQGV IHRDIKSDSI
181 LLTSDGRIKL SDFGFCAQVS KEVPKRKSLV GTPYWMAPEV ISRLPYGTEV DIWSLGIMVI
241 EMIDGEPPYF NEPPLQAMRR IRDSLPPRVK DLHKVSSVLR GFLDLMLVRE PSQRATAQEL
301 LGHPFLKLAG PPSCIVPLMR QYRHH
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Recombinant PAK5 nucleotide sequence:

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  1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
 61 tattttcagg gcgccatgga tccggaattc tccagggtgt cccatgaaca gtttcgggcg
121 gccctgcagc tggtggtcag cccaggagac cccagggaat acttggccaa ctttatcaaa
181 atcggggaag gctcaaccgg catcgtatgc atcgccaccg agaaacacac agggaaacaa
241 gttgcagtga agaaaatgga cctccggaag caacagagac gagaactgct tttcaatgag
301 gtcgtgatca tgcgggatta ccaccatgac aatgtggttg acatgtacag cagctacctt
361 gtcggcgatg agctctgggt ggtcatggag tttctagaag gtggtgcctt gacagacatt
421 gtgactcaca ccagaatgaa tgaagaacag atagctactg tctgcctgtc agttctgaga
481 gctctctcct accttcataa ccaaggagtg attcacaggg acataaaaag tgactccatc
541 ctcctgacaa gcgatggccg gataaagttg tctgattttg gtttctgtgc tcaagtttcc
601 aaagaggtgc cgaagaggaa atcattggtt ggcactccct actggatggc ccctgaggtg
661 atttctaggc taccttatgg gacagaggtg gacatctggt ccctcgggat catggtgata
721 gaaatgattg atggcgagcc cccctacttc aatgagcctc ccctccaggc gatgcggagg
781 atccgggaca gtttacctcc aagagtgaag gacctacaca aggtttcttc agtgctccgg
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901 ctcggacatc cattcttaaa actagcaggt ccaccgtctt gcattgtccc cctcatgaga
961 caatacaggc atcactga
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Reviewed and approved by site quality representative.