

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 Ni²⁺/NTA agarose. Purity 95.8% by SDS-PAGE and Coomassie blue staining. MW = 37.4kDa.

Formulation: 2.067mg/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.

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

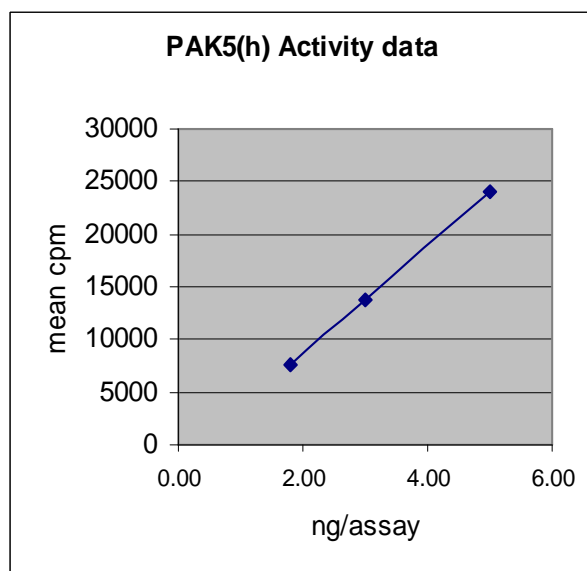
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

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

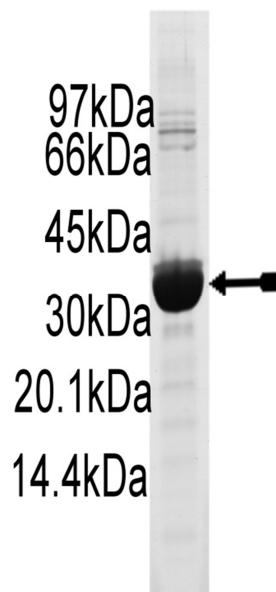
Quality Control Testing

Kinase Assay: 1.81–5.00ng 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.



Certificate of Analysis

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 μ M. Prepare 2mM stock and add 2.5 μ l 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 -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **PAKtide**.
3. Add **2.5 μ l (1.81–5.00ng) PAK5, active**.
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ 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 μ l of 30% phosphoric acid.

Certificate of Analysis

PAK5 Sequence Information

<u>Protein</u>	Human PAK5
<u>Tags</u>	N-Terminal 6His
<u>Native sequence</u>	GenBank NM_020341
<u>Accession number</u>	S31 of recombinant sequence is equivalent to S425 of human PAK5

Recombinant PAK5 amino acid sequence:

```

1  MSYYHHHHHH  DYDIPTTENL  YFQGAMDPEF  SRVSHEQFRA  ALQLVVSPGD  PREYLANFIK
61  IEGSTGIVC   IATEKHTGKQ  VAVKKMDLRK  QQRRELLFNE  VVIMRDYHHD  NVVDMYSSYL
121 VGDELWVVME  FLEGGALTDI  VTHTRMNEEQ  IATVCLSVLR  ALSYLNQGV   IHRDIKSDSI
181 LLTSDGRIKL  SDFGFCAQVS  KEVPKRKS LV  GTPYWMAPEV  ISRLPYGTEV  DIWSLGIMVI
241 EMIDGEPYPF  NEPPLQAMRR  IRDSLPPRVK  DLHKVSSVLR  GFLDLMLVRE  PSQRATAQEL
301 LGHPFLKLAG  PPSCIVPLMR  QYRHH

```

Recombinant PAK5 nucleotide sequence:

```

1  atgtcgtact  accatcacca  tcaccatcac  gattacgata  tccaacgac  cgaaaacctg
61  tattttcagg  ggcgatgga  tccggaattc  tccaggggtg  cccatgaaca  gtttcgggcg
121 gccctgcagc  tgggtggtcag  cccaggagac  cccaggggat  acttggccaa  ctttatcaaa
181 atcggggaag  gctcaaccgg  catcgtatgc  atcgccaccg  agaaacacac  agggaaacaa
241 gtgcagtga  agaaaatgga  cctccggaag  caacagagac  gagaactgct  tttcaatgag
301 gtcgtgatca  tgcgggatta  ccaccatgac  aatgtgggtg  acatgtacag  cagctacctt
361 gtcggcgatg  agctctgggt  ggtcatggag  tttctagaag  gtgggtgcct  gacagacatt
421 gtgactcaca  ccagaatgaa  tgaagaacag  atagctactg  tctgcctgtc  agttctgaga
481 gctctctcct  acctcataa  ccaaggagtg  attcacaggg  acataaaaag  tgactccatc
541 ctctctgaca  gcgatggccg  gataaagtgt  tctgattttg  gtttctgtgc  tcaagtttc
601 aaagagggtgc  cgaagaggaa  atcattggtt  ggactccct  actggatggc  ccctgagggtg
661 atttctaggc  taccttatgg  gacagagggtg  gacatctggt  ccctcgggat  catggtgata
721 gaaatgattg  atggcgagcc  cccctacttc  aatgagcctc  ccctccaggc  gatgctggag
781 atccgggaca  gtttacctcc  aagagtgaag  gacctacaca  aggtttcttc  agtgctccgg
841 ggattcctag  acttgatggt  ggtgagggtg  ccctctcaga  gagcaacagc  ccaggaactc
901 ctccggacatc  cattctaaa  actagcaggt  ccaccgtctt  gcattgtccc  cctcatgaga
961 caatacaggc  atcactga

```

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

© 2014 Eurofins Pharma Discovery Services UK Limited is an independent member of Eurofins Discovery Services.