

Certificate of Analysis

PAK4, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-584, 14-584-K, 14-584M

Parent Lot # WAB0318

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 and N-terminal GST-tagged, recombinant, human PAK4 amino acids 295–end, expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose. Purity 70% by SDS-PAGE and Coomassie blue staining. MW = 61.6kDa.

Specific Activity (Parent lot# WAB0318): 33U/mg, where one unit of PAK4, active activity is defined as 1nmol phosphate incorporated into 0.8mg/ml myelin basic protein per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.83mg/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.

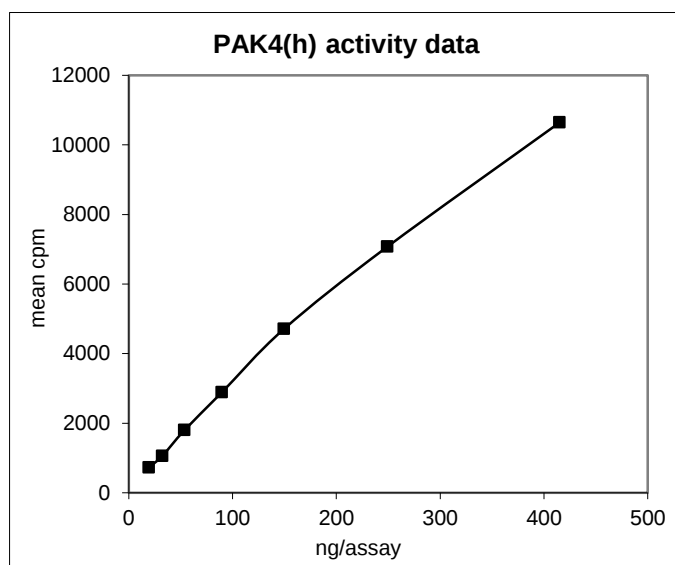
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

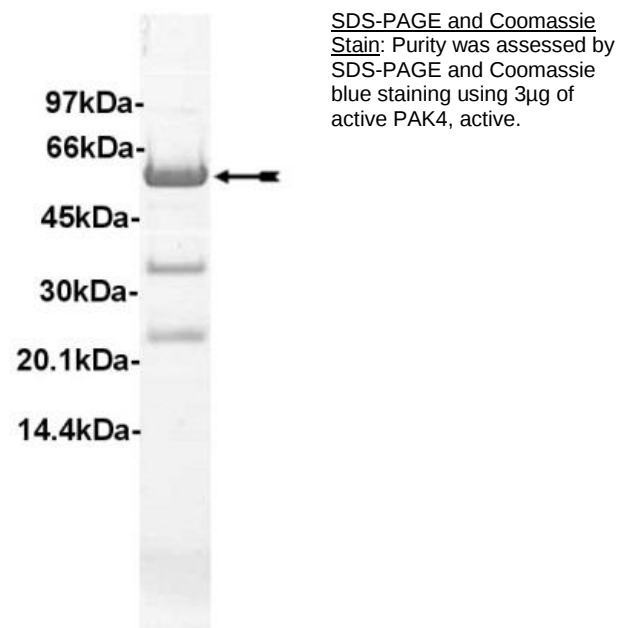
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 19–415ng of this lot of enzyme phosphorylated 0.8mg/ml myelin basic protein (MBP) 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 PAK4 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. **Substrate: Myelin Basic Protein (MBP):** Use at a final assay concentration of 0.8mg/ml. Make up an 8.0mg/ml stock. Use 2.5µl of stock solution per assay point.
3. **PAK4, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1 mg/ml BSA. Use 19–415ng 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 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **myelin basic protein (MBP)**.
3. Add **2.5µl (19–415ng) PAK4, active**.
4. Add 5µl of dH₂O
5. Add 10µl of [γ -³³P]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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Sequence Information

Protein	Human PAK4
Tags	N-terminal 6His and N-terminal GST tags
Native sequence	E242 of recombinant sequence is equivalent to E295 of native human PAK4
Accession number	GenBank NM_005884

Recombinant PAK4 amino acid sequence:

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1  MHHHHHHASP MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL
61  EFPNLPYYID GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA
121 YSKDFETLKV DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL
181 DAFPKLVCFK KRIEAIPOID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFGPLG
241 SEPQRVSHEQ FRAALQLVVD PGDPRSYLDN FIKIGEGSTG IVCIAVRSS GKLVAVKKMD
301 LRKQQRRELL FNEVVIMRDY QHENNVEMYN SYLVGDELWV VMEFLEGGAL TDIVTHTRMN
361 EEQIAAVCLA VLQALSVLHA QGVIHRDIKS DSILLTHDGR VKLSDFGFCA QVSKEVPRRK
421 SLVGTPYWMA PELISRLPYG PEVDIWSLGI MVIEMVDGEP PYFNEPPLKA MKMIRDNLPP
481 RLKLNHKKVSP SLKGFLLDRLL VRDPAQRATA AELLKHPFLA KAGPPASIVP LMRQNRTR

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Recombinant PAK4 nucleotide sequence:

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1  atgcaccatc accatcacca tgctagtccg atgtccccta tactaggtta ttggaaaatt
61  aagggccttg tgcaaccac tgcacttctt ttggaatatc ttgaagaaaa atatgaagag
121 catttgatat agcgcgatga aggtgataaa tggcgaaaca aaaagtttga attgggtttg
181 gagtttccca atcttcctta ttatatgat ggtgatgta aattaacaca gtctatggcc
241 atcatacgtt atatatctga caagcacaa atgttgggtg gttgtccaaa agagcgtgca
301 gagatttcaa tgcttgaagg agcggttttg gatattagat acggtgtttc gagaattgca
361 tatagtaaag actttgaaac tctcaaagtt gattttctta gcaagctacc tgaatgctg
421 aaaatgttcg aagatcgttt atgtcataaa acatatatta atggtgatca tgaacccat
481 cctgacttca tgttgatatg cgctcttgat gttgttttat acatggaccc aatgtgcctg
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1561 aaggcagggc cgcctgccag catcgtgccc ctcatgcgcc agaaccgcac cagatag

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