

Certificate of Analysis

PAK1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-927, 14-927-K, 14-927M

Parent Lot # D12NP022N

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 FLAG-tagged, recombinant, human PAK1 amino acids 150-end, expressed by baculovirus in Sf21 insect cells. Purified by immunoaffinity chromatography. Purity 74% by SDS-PAGE and Coomassie blue staining. MW = 46kDa.

Formulation: 0.170mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 10mM β -glycerophosphate, 1mM sodium orthovanadate, 1mM NaF, 28% v/v glycerol, 0.03% Brij-35, 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.

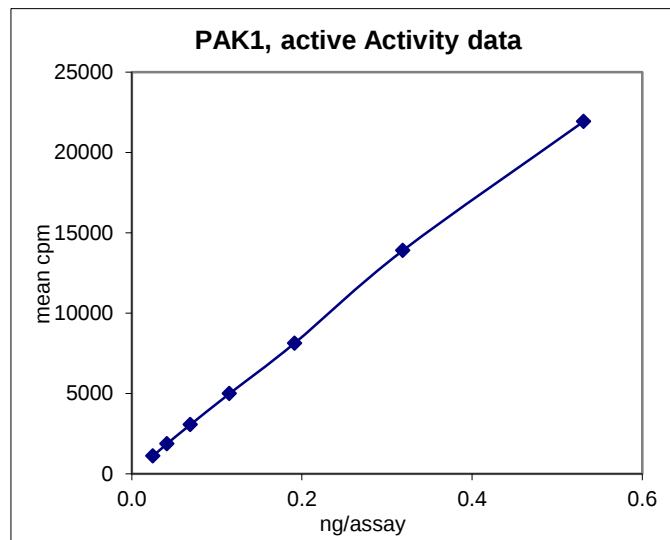
Specific Activity (Parent lot# D12NP022N): 45501U/mg, where one unit of PAK1, active activity is defined as 1nmol phosphate incorporated into 200 μ M (RRRLSFAEPG) per minute at 30°C with a final ATP concentration of 100 μ M.

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.

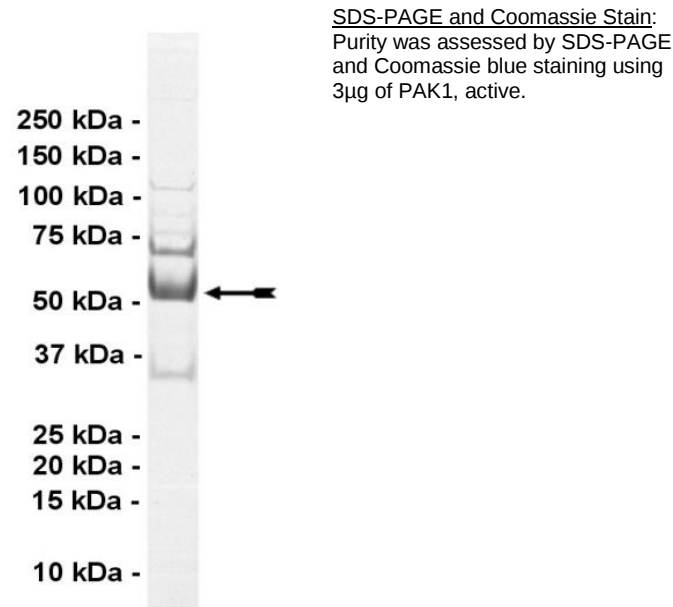
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.02–0.53ng of this lot of enzyme phosphorylated 200 μ M (RRRLSFAEPG) 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 PAK1 with the translated 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 EDT.
2. **(RRRLSFAEPG):** Use at a final assay concentration of 200 μ M. Prepare a 2mM stock and add 2.5 μ l of stock per assay point.
3. **PAK1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.02–0.53ng per assay point.
4. **[γ -³³P]ATP:** 2.5 x MgAc/[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 **(RRRLSFAEPG)**.
3. Add **2.5 μ l (0.02–0.53ng) PAK1, 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.

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PAK1 Sequence Information

<u>Protein</u>	human PAK1
<u>Tags</u>	N-terminal FLAG
<u>Native sequence</u>	A16 of the recombinant protein is equivalent to A150 of human PAK1
<u>Accession number</u>	GenBank NM_002576

Recombinant PAK1 amino acid sequence:

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1  MGGDYKDDDD KDPEFAEDYN SSNALNVKAV SETPAVPPVS EDEDDDDDDA TPPPVIAPRP
61  EHTKSVYTRS VIEPLPVTPT RDVATSPISP TENNTTPPDA LTRNTEKQKK KPKMSDEEIL
121 EKLRSIVSVG DPKKKYTRFE KIGQGASGTV YTAMD VATGQ EVAIKQMN LQ QQPKKELIIN
181 EILVMRENKN PNIVNYLDSY LVGDELWVVM EYLAGGSLTD VVTETCMDEG QIAAVCRECL
241 QALEFLHSNQ VIH RDIKSDN ILLGMDGSVK LTDFGFCAQI TPEQSKRSTM VGTPYWMAPE
301 VVTRKAYGPK VDIWSLGIMA IEMIEGEPY LNENPLRALY LIATNGTPEL QNPEKLSAIF
361 RDFLNRCLEM DVEKRGSAKE LLQHQLKIA KPLSSLTPLI AAAKEATKNN H

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Recombinant PAK1 nucleotide sequence:

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1  atgggaggtg actacaaaga cgatgacgac aaggatccgg aattcgctga ggattacaat
61  tcttctaata ccttgaatgt gaaggctgtg tctgagactc ctgcagtgcc accagtttca
121 gaagatgagg atgatgatga tgatgatgct accccaccac cagtgattgc tccacgcca
181 gagcacacaa aatctgtata cacacggtct gtgattgaac cacttcctgt cactccaact
241 cgggacgtgg ctacatctcc catttcacct actgaaaata acaccactcc accagatgct
301 ttgaccggga atactgagaa gcagaagaag aagcctaata tgtctgatga ggagatcttg
361 gagaaattac gaagcatagt gagggtgggc gatcctaaga agaaatatac acggtttgag
421 aagattggac aagggtgctt aggcaccgtg tacacagcaa tggatgtggc cacaggacag
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601 ctctgtggag atgagctgtg ggttggttat gaatacttgg ctggaggctc cttgacagat
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1141 ctgctacagc atcaattcct gaagattgcc aagcccctct ccagcctcac tccactgatt
1201 gctgcagcta aggaggcaac aaagaacaat cactaa

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