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Certificate of Analysis

PEK, active

(Recombinant enzyme expressed in Sf21 cells)
Item # 14-916, 14-916-K, 14-916M
Parent Lot # 2042626

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 PEK amino acids 536 – end, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography. Purity 81.7% by SDS-PAGE and Coomassie blue staining. MW = 71kDa.

Specific Activity (Parent lot# 2042626): 383U/mg, where one unit of PEK, active activity is defined as 1nmol phosphate incorporated into 300µM RSRSRSRSRSRSRSR per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.82mg/ml of enzyme in of 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

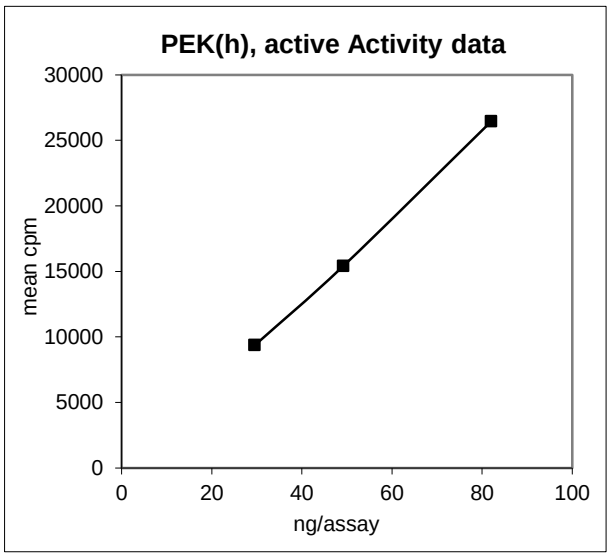
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

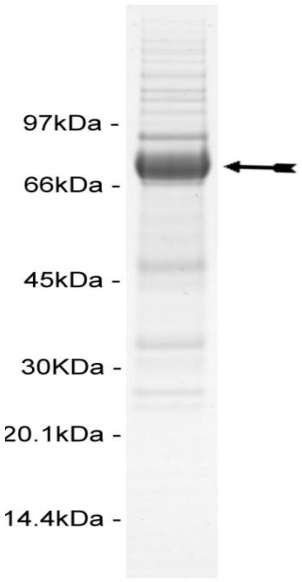
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 30–82ng of this lot of enzyme phosphorylated 300µM RSRSRSRSRSRSRSR 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 PEK with the translated sequence listed on page three.



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

Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom
T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA, other components.
2. **RSRSRSRSRSRSRSR**: Use at a final assay concentration of 300µM. Prepare a 3mM stock and add 2.5µl of stock per assay point.
3. **PEK(h), active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 30–82ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x MgAc/[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 **RSRSRSRSRSRSRSR**.
3. **Add 2.5µl (30–82ng) PEK(h), 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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PEK Sequence Information

Protein Human PEK

Tags *N*-terminal 6His

Native sequence T37 of the recombinant protein is equivalent to T536 of human PEK

Accession number GenBank NM_004836

Recombinant PEK amino acid sequence:

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  1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF KGLRRQTTFI VRRLFHPHPH RQRKESETQC
 61 QTENKYDSVS GEANDSSWND IKNSGYISRY LTDFEPIQCL GRGGFGVVFE AKNKVDDCNY
121 AIKRIRLPNR ELAREKVMRE VKALAKLEHP GIVRYFNAWL EAPPEKWQEK MDEIWLKDES
181 TDWPLSSPSP MDAPSVKIRR MDPFATKEHI EIIAPSPQRS RSFSVGISCD QTSSSESQFS
241 PLEFSGMDHE DISESVDAAY NLQDSCLTDC DVEDGTMDGN DEGHSFELCP SEASPYVRSR
301 ERTSSSIVFE DSGCDNASSK EEPKTNRLHI GNHCANKLTA FKPTSSKSSS EATLSISPPR
361 PTTLSLDLTK NTTEKLQPSS PKVYLYIQMQ LCRKENLKDW MNGRCTIEER ERSVCLHIFL
421 QIAEAVEFLH SKGLMHRDLK PSNIFFTMDD VVKVGDFGLV TAMDQDEEEQ TVLTPMPAYA
481 RHTGQVGTKL YMSPEQIHGN SYSHKVDIFS LGLILFELLY PFSTQMERVR TLTDVRNLKF
541 PPLFTQKYPC EYVMVQDMLS PSPMERPEAI NIIENAVFED LDFPGKTVLR QRSRSLSSSG
601 TKHSRQSNNS HSPLPSN
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Recombinant PEK nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatgga tccggaattc aaaggcctac gtcgacaaac aacgtttatt
 121 gtgcgcaggc ttttccatcc tcatcctcac aggcaaagga aggagtctga aactcagtgt
 181 caaactgaaa ataaatatga ttctgtaagt ggtgaagcca atgacagtag ctggaatgac
 241 ataaaaaact ctggatatat atcacgatat ctaactgatt ttgagccaat tcaatgcctg
 301 ggacgtggtg gctttggagt tgtttttgaa gctaaaaaca aagtagatga ctgcaattat
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 781 aacctccagg acagttgcct tacagactgt gatgtggaag atgggactat ggatggcaat
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1141 ccaaaggtgt atctttacat tcaaatgcag ctgtgcagaa aagaaaacct caaagactgg
1201 atgaatggac gatgtaccat agaggagaga gagaggagcg tgtgtctgca catcttcctg
1261 cagatcgcag aggcagtgga gtttcttcac agtaaaggac tgatgcacag ggacctcaag
1321 ccatccaaca tattctttac aatggatgat gtggtcaagg ttggagactt tgggttagtg
1381 actgcaatgg accaggatga ggaagagcag acggttctga ccccaatgcc agcttatgcc
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1621 ccaccattat ttactcagaa atatccttgt gagtacgtga tggttcaaga catgctctct
1681 ccatccccca tggaacgacc tgaagctata aacatcattg aaaatgctgt atttgaggac
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1741 ttggactttc caggaaaaac agtgctcaga cagaggtctc gctccttgag ttcatcggga
1801 acaaaacatt caagacagtc caacaactcc catagccctt tgccaagcaa ttag
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Reviewed and approved by site quality representative.