

Certificate of Analysis

PRK2, active

(Recombinant enzyme expressed in Sf21 cells)

Item # 14-549, 14-549-K, 14-549M

Parent Lot # 1590299

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: Recombinant human PRK2, residues 501–end, containing N-terminal 6His tag. Expressed by baculovirus in Sf21 insect cells and purified by Ni²⁺/NTA-agarose. Purity 71.9% by SDS-PAGE and Coomassie blue staining. MW = 59.9kDa.

Specific Activity (Parent lot# 1590299): 202U/mg, where one unit equals the incorporation of 1nmol of phosphate per min into 30µM synthetic substrate peptide (AKRRRLSSLRA) at 30°C with a final ATP concentration of 100µM.

Formulation: : 0.63mg/ml of enzyme in 50mM Tris/HCl pH7.5, 270mM sucrose, 150mM NaCl, 1mM benzamidine, 0.1mM EGTA, 0.1 % 2-mercaptoethanol, 0.03% Brij-35.

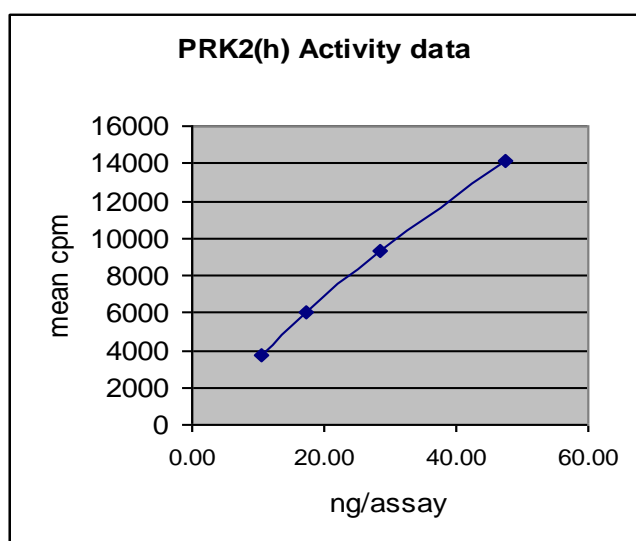
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

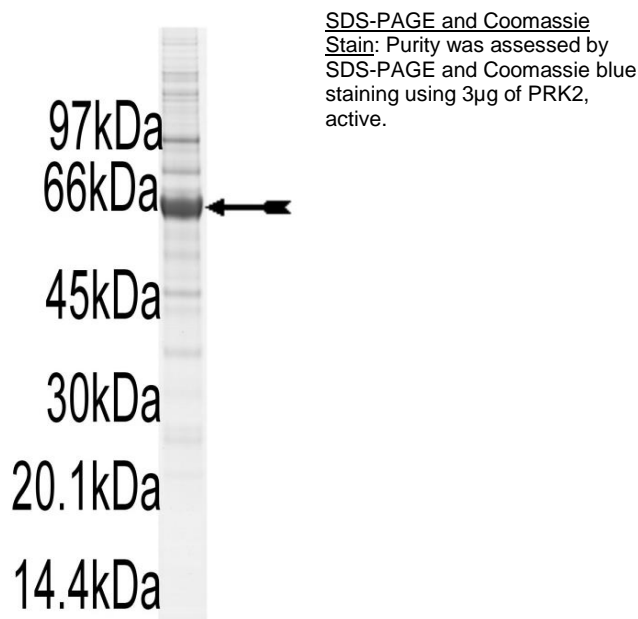
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 10–47ng of this lot of enzyme phosphorylated 0.33mg/ml synthetic substrate peptide (AKRRRLSSLRA) 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 PRK2, active with the translated sequence listed on page three.



Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

- 1. 10 x Reaction Buffer:** 500mM Tris/HCl pH7.5, 0.1mM EGTA, 1 % 2-mercaptoethanol.
- 2. PRK2, active:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1% 2-mercaptoethanol, 1mg/ml BSA.
- 3. [γ -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 2mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)
- 4. PRK2 synthetic substrate peptide (AKRRRLSSLRA), active:** Use a final assay concentration of 30 μ M. Make up a 300 μ M stock. Add 2.5 μ l of stock solution. Use 10–47ng per assay point.

Assay Procedure (96 well plate format):

1. Add 2.5 μ l of reaction buffer per assay to wells
2. Add 2.5 μ l of **substrate peptide**.
3. Add **2.5 μ l (10–47ng) 6His-PRK2 (hu,501-end), active**.
4. Make up to 15 μ l with dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Spot a 20 μ l aliquot onto the centre of a 2cm x 2cm **P81 paper square**.
8. Wash the assay squares twice for 5 minutes with 75mM phosphoric acid.
9. Wash the assay squares once for 2 minutes with acetone.
10. Transfer the assay squares to a vial and add 1ml of scintillation cocktail.
11. 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

PRK2 Sequence Information

<u>Protein</u>	Human PRK2, 501–end
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	G40 of the recombinant protein equivalent to G501 of the native sequence.
<u>Accession number</u>	GenBank NM_006256

Recombinant amino acid sequence:

```

1 MSYHHHHHHH DYDIPTTENL YFQGAMGSAT MDYKDDDDKG KTFLRAPQMN INIATWGRLV
61 RRAIPTVNHS GTFSPQAPVP TTVPVVDVRI PQLAPPASDS TVTKLDFDLE PEPAPPAPRA
121 SSLGEIDESS ELRVLDPGQ DSETVFDIQN DRNSILPKSQ SEYKPDTPQS GLEYSGIQEL
181 EDRRSQORFQ FNLQDFRCCA VLGRGHFGKV LLAEYKNTNE MFAIKALKKG DIVARDEVDS
241 LMCEKRIFET VNSVRHPFLV NLFACFQKE HVCFVMEYAA GGDLMHIIHT DVFSEPRAVF
301 YAACVVLGLQ YLHEHKIVYR DLKLDNLLLD TEGFVKIADF GLCKEKMGGYD DRTSTFCGTP
361 EFLAPEVLTE TSYTRAVDWW GLGVLIYEML VGESPPFGDD EEEVFDIVN DEVRYPRFLS
421 TEAISIMRRL LRRNPERRLG ASEKDAEDVK KHPFFRLIDW SALMDKKVKP PFIPTIRGRE
481 DVSNFDEFT SEAPILTPPR EPRILSEEEQ EMFRDFDYIA DWC
  
```

Recombinant nucleotide sequence:

```

1 atgtcgact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatggg atccgccacc atggactaca aggacgacga tgacaagggc
121 aaaacatttc tcagagctcc tcaaatgaat attaatttg ccacttgggg aaggctagta
181 agaagagcta ttcctacagt aatcattct ggacacctca gccctcaagc tcctgtgcct
241 actacagtgc cagtggttga tgtacgcac cctcaactag cacctccagc tagtgattct
301 acagtaacca aattggactt tgatcttgag cctgaacctc ctccagcccc accacgagct
361 tcttctcttg gagaaataga tgaatcttct gaattaagag ttttgatat accaggacag
421 gattcagaga ctgtttttga tattcagaat gacagaaata gtatacttcc aaaatctcaa
481 tctgaataca agcctgatac tcctcagtca ggccatgaat atagtggtat tcaagaactt
541 gaggacagaa gatctcagca aaggtttcag ttaactctac aagatttcag gtgtttgtgct
601 gtcttgggaa gaggacattt tggaaagggt ctttagctg aatataaaaa cacaaatgag
661 atgtttgcta taaaagcctt aaagaaagga gatattgtgg ctcgagatga agtagacagc
721 ctgatgtgtg aaaaaagaat ttttgaact gtgaatagtg taaggcatcc ctttttggtg
781 aaccttttg catgtttcca aaccaagag catgtttgct ttgtaatgga atatgctgcc
841 ggtggggacc taatgatgca cattcactat gatgtctttt ctgaaccaag agctgtatct
901 tatgctgctt gtgtagtctt tgggttcgag tttttacatg aacacaaaat tgtttataga
961 gatttgaat tggataactt attgctagat acagagggct ttgtgaaaat tgctgatttt
1021 ggtctttgca aagaaggaat gggatatgga gatagaacaa gcacattttg tggcactcct
1081 gaatttcttg cccagaagt attaacagaa acttcttata caagggtgtg agattggtgg
1141 ggcttggcg tgcttatata tgaatgctt gttggtgagt ctccctttc tggatgatgat
1201 gaagaggaag ttttgacag tattgtaaat gatgaagtaa ggtatccaag gttcttatct
1261 acagaagcca tttctataat gagaaggctg ttaagaagaa atcctgaacg ggccttggg
1321 gctagcgaga aagatgcaga ggatgtaaaa aagcacccat tttccggct aattgattgg
1381 agcgtcttga tggacaaaaa agtaaagcca ccatttatac ctaccataag aggacgagaa
1441 gatgttagta attttgatga tgaatttacc tcagaagcac ctattctgac tccacctgca
1501 gaaccaagga tactttcgga agaggagcag gaaatgttca gagattttga ctacattgct
1561 gattggtggt aa
  
```

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.