

Certificate of Analysis

Pyk2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-567, 14-567-K, 14-567M

Parent Lot # WAC0540

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, full-length, human Pyk2, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity 59% by SDS-PAGE and Coomassie blue staining. MW = 117.2kDa.

Specific Activity (Parent lot# WAC0540): 25U/mg, where one unit of Pyk2, active activity is defined as 1nmol phosphate incorporated into 0.1mg/ml poly(Glu, Tyr) (4:1) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.00mg/ml of enzyme in Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

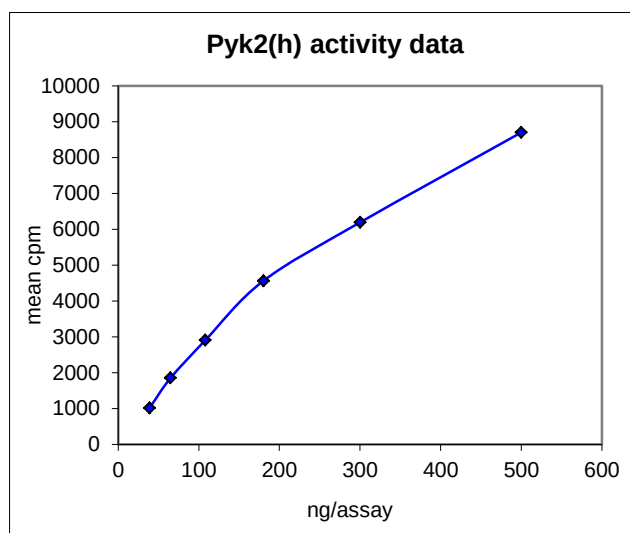
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

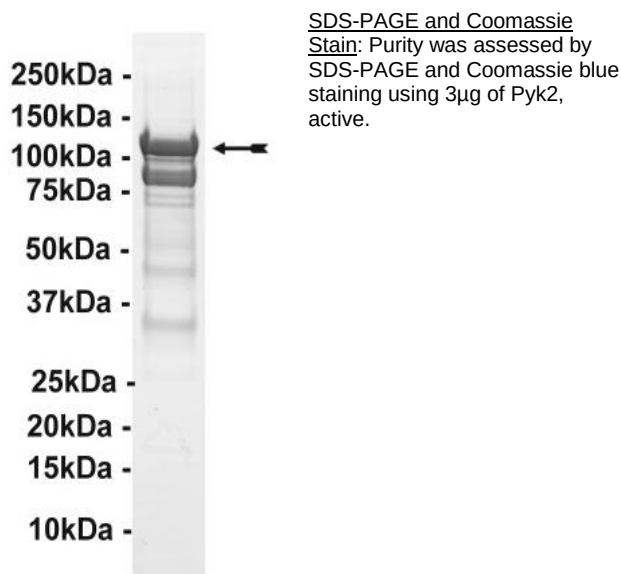
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 38.88–500ng of this lot of enzyme phosphorylated 0.1mg/ml poly(Glu, Tyr) (4:1) 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 Pyk2 with the translated native sequence listed on page three.



Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Poly (Glu, Tyr) (4:1):** Use at a final assay concentration of 0.1mg/ml. Prepare a 1mg/ml stock and add 2.5µl of stock per assay point.
3. **Pyk2, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1 mg/ml BSA. Use 38.88–500ng 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 **poly(Glu, Tyr) (4:1)**.
3. Add **2.5µl (38.88–500ng) Pyk2, active**.
4. Add 5µl of dH₂O.
5. Add 10µl of diluted [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 **Filtermat A**.
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.

Certificate of Analysis

Pyk2 Sequence Information

<u>Protein</u>	Human Pyk2
<u>Tags</u>	N-Terminal 6His
<u>Native sequence</u>	M10 of the recombinant protein is equivalent to M1 of human Pyk2
<u>Accession number</u>	GenBank S80542

Recombinant Pyk2 amino acid sequence:

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1  MHHHHHHEFM  SGVSEPLSRV  KLGTLRRPEG  PAEPMVVVPV  DVEKEDVRIL  KVCFYNSFSN
61  PGKNFKLVKC  TVQTEIREII  TSILLSGRIG  PNIRLAECYG  LRLKHKMSDE  IHWLHPQMTV
121  GEVQDKYECL  HVEAEWRYDL  QIRYLPEDFM  ESLKEDRTTL  LYFYQQLRND  YMQRYASKVS
181  EGMALQLGCL  ELRRFFKDMP  HNALDKKSNF  ELLEKEVGLD  LFFPKQMQUEN  LKPKQFRKMI
241  QQTFQQYASL  REEECVMKFF  NTLAGFANID  QETYRCELIQ  GWNITVDLVI  GPKGIRQLTS
301  QDAKPTCLAE  FKQIRSIRCL  PLEEGQAVLQ  LGIEGAPQAL  SIKTSSLAEA  ENMADLIDGY
361  CRLQGEHQGS  LIIHPRKDGE  KRNSLPQIPM  LNLEARRSHL  SESCOIESDI  YAEIPDETLR
421  RPPGPQYGIA  REDVVLNRIL  GEGFFGEVYE  GVYTNHKGEK  INVAVKTCKK  DCTLDNKEKF
481  MSEAVIMKNL  DHPHIVKLIG  IIEEPTWII  MELYPYGELG  HYLERKNKSL  KVLTLVLYSL
541  QICKAMAYLE  SINCVRDIA  VRNILVASPE  CVKLGDGFLS  RYIEDEDYK  ASVTRLPIKW
601  MSPESINFRR  FTTASDVWMF  AVCMWEILSF  GKQPFWLEN  KDVIGVLEKG  DRLPKPDLCP
661  PVLYTLMTRC  WDYDPSDRPR  FTELVCSLSD  VYQMEKDIAM  EQERNARYRT  PKILEPTAFQ
721  EPPPKPSRPK  YRPPQTNLL  APKLQFQVPE  GLCASSPTLT  SPMEYPSPVN  SLHTPPLHRH
781  NVFKRHSRE  EDFIQSSRE  EAQQLWEAEK  VKMRQILDQK  QKQMVEDYQW  LRQEEKSLDP
841  MVYMNDSPL  TPEKEVGYLE  FTGPPQKPPR  LGAQSIQPTA  NLDRTDDLVI  LNMELVRVAV
901  LELKNELCQL  PPEGYVVVK  NVGLTLRKLI  GSVDDLPSL  PSSSRTEIEG  TQKLLNKDLA
961  ELINKMRLAQ  QNAVTSLSSE  CKRQMLTASH  TLAVDAKNLL  DAVDQAKVLA  NLAHPPEAE
  
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Recombinant Pyk2 nucleotide sequence:

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121  gatgtggaaa  aggaggacgt  gcgtatcctc  aaggtctgct  tctatagcaa  cagcttcaat
181  cctgggaaaa  acttcaaact  ggtcaaatgc  actgtccaga  cggagatccg  ggagatcatc
241  acctccatcc  tgctgagcgg  gcggatcggg  cccaacatcc  ggttggtcta  gtgctatggg
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