

Certificate of Analysis

PTK5, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-693, 14-693-K, 14-693M

Parent Lot # 31057U

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 PTK5, amino acids 218–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 61.6% by SDS-PAGE and Coomassie blue staining. MW = 38kDa.

Specific Activity (Parent lot# 31057U): 1130U/mg, where one unit of PTK5, active activity is defined as 1nmol phosphate incorporated into 500µM (GGESEYFELVKKKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.283mg/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.

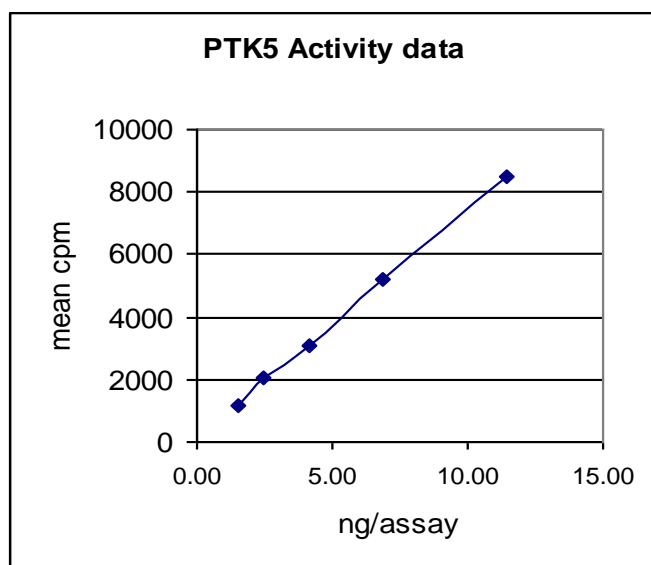
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

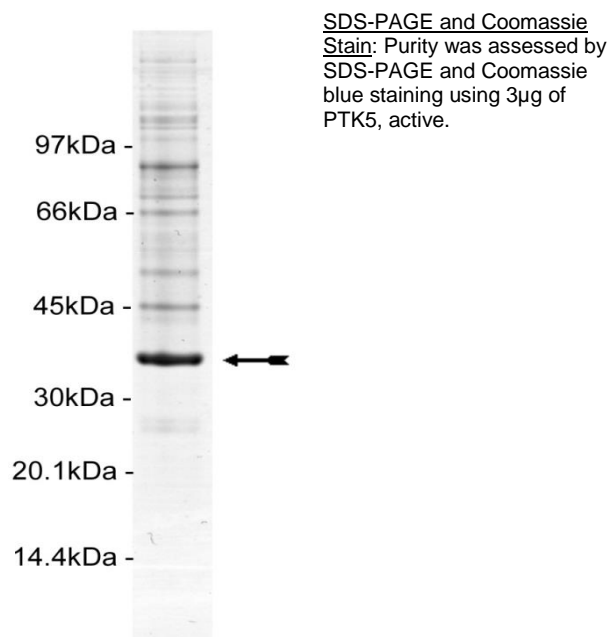
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 1.5–11.4ng of this lot of enzyme phosphorylated 500µM (GGESEYFELVKKKK) 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 PTK5, 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. **(GGEEEEYFELVKKKK):** Use at a final assay concentration of 500 μ M. Prepare a 5mM stock and add 2.5 μ l of stock per assay point.
3. **PTK5, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.5–11.4ng 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 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **(GGEEEEYFELVKKKK)**.
3. Add **2.5 μ l (1.5–11.4ng) PTK5, 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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PTK5 Sequence Information

<u>Protein</u>	Human PTK5
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	D37 of the recombinant protein is equivalent to D218 of human PTK5
<u>Accession number</u>	GenBank NM_002031

Recombinant PTK5 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF KGLRRPDLSY KTVDQWEIDR NSIQLLKRLG
61 SGQFGEVWEG LWNNTTPVAV KTLKPGSMDP NDFLREQIM KNLRHPKLIQ LYAVCTLEDP
121 IYIITELMRH GSLQEYLQND TGSKIHLTQQ VDMAAQVASG MAYLESRNYI HRDLAARNVL
181 VGEHNIYKVA DFGLARVFKV DNEDIYESRH EIKLPVKWTA PEAIRSNKFS IKSDVWSFGI
241 LLYEIITYGK MPYSGMTGAQ VIQMLAQNYR LPQPSNCPQQ FYNIMLECWN AEPKERPTFE
301 TLRWKLEDYF ETDSSYS DAN NFIR

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Recombinant PTK5 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc aaaggcctac gtcgaccgga tttgtcgatat
121 aaaaccgtgg accaatggga gatagaccgc aactccatac agcttctgaa gcgattggga
181 tctggtcagt ttggcgaagt atgggaaggt ctgtggaaca ataccactcc agtagcagtg
241 aaaacattaa aaccaggttc aatggatcca aatgacttcc tgagggaggc acagataatg
301 aagaacctaa gacatccaaa gcttatccag ctttatgctg tttgcacttt agaagatcca
361 atttatatta ttacagagtt gatgagacat ggaagtctgc aagaatatct ccaaaatgac
421 actggatcaa aaatccatct gactcaacag gtagacatgg cggcacaggt tgcctctgga
481 atggcctatc tggagtctcg gaactacatt cacagagatc tggctgccag aaatgtcctc
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601 gataatgaag acatctatga atctagacac gaaataaagc tgccggtgaa gtggactgcg
661 cccgaagcca ttcgtagtaa taaattcagc attaagtccg atgtatggtc atttggaatc
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961 aacttcataa gatga

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