

Certificate of Analysis

Pim-1, active

(Recombinant enzyme expressed in *E. coli* cells)

Item # 14-573, 14-573-K, 14-573M

Parent Lot # 2263618

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 GST-tagged recombinant human full length Pim-1, expressed in *E. coli* cells. Purified using glutathione agarose. Purity 85.4% by SDS-PAGE and Coomassie blue staining. MW = 62kDa.

Formulation: 1.872mg/ml of enzyme in 50mM Tris/HCl pH7.5, 0.1mM EGTA, 150mM NaCl, 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 6 months 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# 2263618): 3495U/mg, where one unit of Pim-1 activity is defined as 1nmol phosphate incorporated into 100µM (KKRNRTLTV) per minute at 30°C with a final ATP concentration of 100µM.

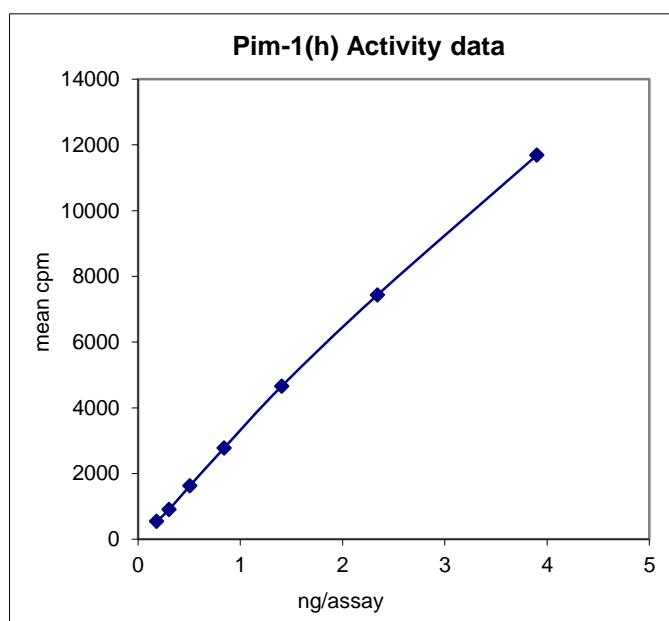
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

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

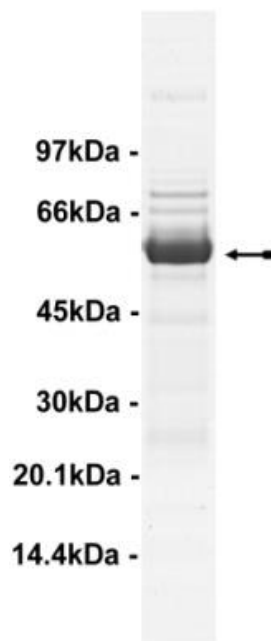
Quality Control Testing

Kinase Assay: 0.2–3.9ng of this lot of enzyme phosphorylated 100µM (KKRNRTLTV) 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 Pim-1 with the translated native sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of Pim-1, active.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(KKRNRTLTV):** Use at a final assay concentration of 100 μ M. Prepare a 1mM stock and add 2.5 μ l of stock per assay point.
3. **Pim-1, active:** Dilute with 20mM MOPS/NaOH, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.2–3.9ng 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 **(KKRNRTLTV)**.
3. Add **2.5 μ l (0.2–3.9ng) Pim-1, 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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Pim-1 Sequence Information

<u>Protein</u>	human Pim-1
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M230 of the recombinant protein is equivalent to M1 of human Pim-1
<u>Accession number</u>	GenBank M27903

Recombinant Pim-1 amino acid sequence:

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1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQ SMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLP EML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAI PQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSPEFM LLSKINSLAH
241 LRAAPCNDLH ATKLAPGKEK EPLESQYQVG PLLGSGGFGS VYSGIRVSDN LPVAIKHVEK
301 DRISDWGELP NGTRVPMEVV LLKKVSSGFS GVIRLLD WFE RPDSFVLILE RPEPVQDLFD
361 FITERGALQE ELARFFWQV LEAVRHCHNC GVLHRDIKDE NILIDLNRGE LKLIDFGSGA
421 LLKDTVYTDF DGTRVYSPPE WIRYHRYHGR SAAVWSLGIL LYDMVCGDIP FEHDEEIIIRG
481 QVFFRQRVSS ECQHLIRWCL ALRPSDRPTF EEIQNHPPWMO DVLLPQETAE IHLHSLSPGP
541 SK

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Recombinant Pim-1 nucleotide sequence:

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1 atgtccccta tactagg tta ttgaaaatt aaggccttg tgcaaccac tcgacttctt
61 ttggaatatt ttgaagaaaa atatgaagag catttgatg agcgcgatga aggtgataaa
121 tggcgaaaca aaaagt t tga attgggttg gagtttcca atcttcctta ttatattgat
181 ggtgatg tta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
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1501 gccctgagac catcagatag gccaaccttc gaagaaatcc agaaccatcc atggatgcaa
1561 gatgttctcc tgcccagga aactgctgag atccacctcc acagcctgtc gccggggccc
1621 agcaaatag

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