

Certificate of Analysis

PIM2, active

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

Item # 14-607, 14-607-K, 14-607M

Parent Lot # 1872440

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 PIM2, 2-end, expressed in *E. coli* cells. Purified using glutathione agarose. Purity 36.8% by SDS-PAGE and Coomassie blue staining. MW = 63.5kDa.

Specific Activity (Parent lot# 1872440): 107U/mg, where one unit of PIM2, active activity is defined as 1nmol phosphate incorporated into 300µM PIM2tide (cat# 12-542) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.833mg/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.

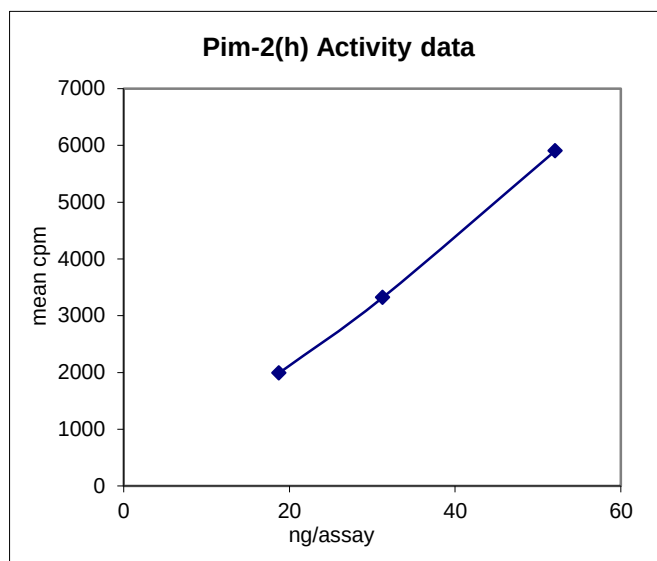
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

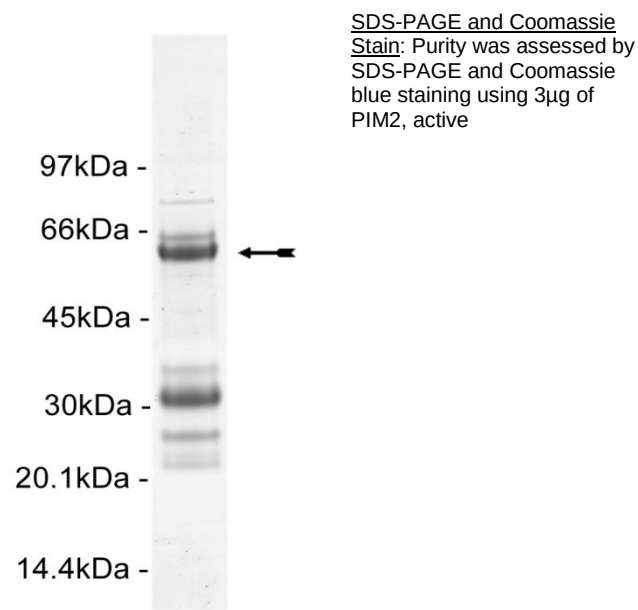
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 19–52ng of this lot of enzyme phosphorylated 300µM PIM2tide (cat# 12-542) 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 as PIM2 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. **PIM2tide (Catalogue# 12-542):** Use at a final assay concentration of 300µM. Make up a 3mM stock. Add 2.5µl of stock per assay point.
3. **PIM2, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 19–52ng 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 **PIM2tide**.
3. Add 2.5µl (**19–52ng**) **PIM2, 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 **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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PIM2 Sequence Information

<u>Protein</u>	Human PIM2
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	L232 of the recombinant protein is equivalent to L2 of human PIM2
<u>Accession number</u>	GenBank U77735

Recombinant PIM2 amino acid sequence:

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1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGPLG SLTKPLQGPP
241 APPGTPTPPP GGDREAFEA EYRLGPLLGK GFGTVFAGH RLTDRLQVAI KVIPRNRVLG
301 WSPLSDSVTC PLEVALLWKV GAGGGHPGVI RLLDWFETQE GFMLVLERPL PAQDLFDYIT
361 EKGPLGEGPS RCFFGQVVAA IQHCHSRGVV HRDIKDENIL IDLRRGCAKL IDFGSGALLH
421 DEPYTDFDGT RVYSPPEWIS RHQYHALPAT VWSLGILLYD MVCGDIPFER DQEILEAELH
481 FPAHVSPDCC ALIRRCLAPK PSSRPSLEEI LLDPWMQTPA EDVTPQPLQR RPCPFLVLA
541 TLSLAWPGLA PNGQKSHPM MSQG

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Recombinant PIM2 nucleotide sequence:

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1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61 ttggaatata ttgaagaaaa atatgaagag catttgatat agcgcgatga aggtgataaa
121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatatgtat
181 ggtgatgtta aattaacaca gtctatggcc atcatagctt atatagctga caagcacaac
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
301 gatattagat acggtgtttc gagaattgca tataagtaaag actttgaaac tctcaaagtt
361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
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721 gcgcccccg ggacccccac gccgccgcca ggaggcaagg atcggaagc gttcaggacc
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1201 atagacctac gccgtggctg tgccaaactc attgattttg gttctggtgc cctgcttcat
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1321 cgacaccagt accatgactt cccggccact gtctggtcac tgggcacatc cctctatgac
1381 atggtgtgtg gggacattcc ctttgagagg gaccaggaga ttctggaagc tgagctccac
1441 ttcccagccc atgtctccc agactgctgt gccctaacc gccggtgcct ggcccccaaa
1501 cttcttccc gacctcact ggaagagatc ctgctggacc cctggatgca aacaccagcc
1561 gaggatgtta cccctcaacc cctccaaagg aggccctgcc ctttggcct ggtccttgct

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1621 accctaagcc tggcctggcc tggcctggcc cccaatgggc agaagagcca tcccatggcc
1681 atgtcacagg gatag

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