

Certificate of Analysis

Pim-3, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-738, 14-738-K, 14-738M

Parent Lot # 33220U

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 Pim-3, 2–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 80% by SDS-PAGE and Coomassie blue staining. MW = 39.6kDa.

Specific Activity (Parent lot# 33220U): 5210U/mg, where one unit of Pim-3, active activity is defined as 1nmol phosphate incorporated into 300µM (RSRHSSYPAGT) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.05mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamide, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

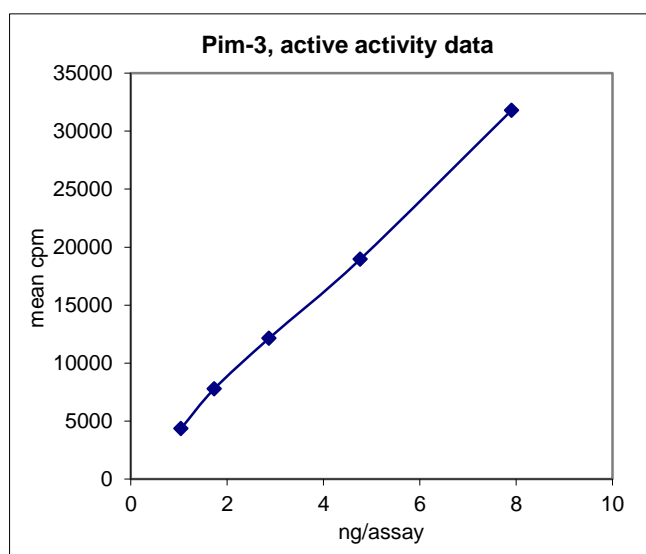
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

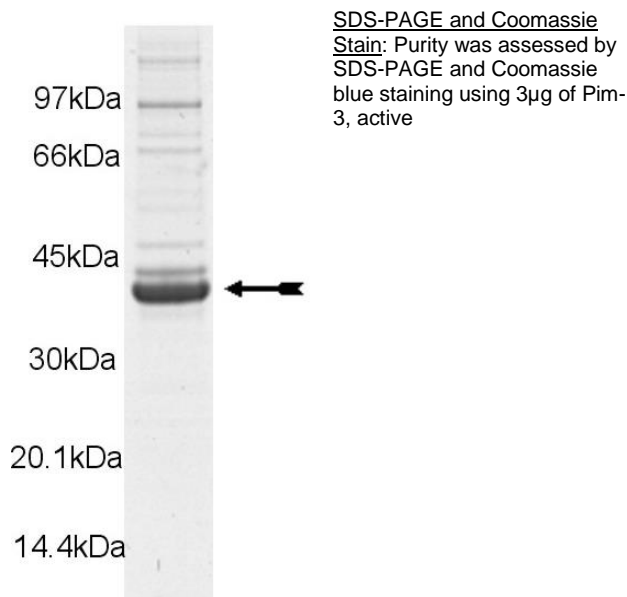
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 1–8ng of this lot of enzyme phosphorylated 300µM (RSRHSSYPAGT) 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 Pim-3 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. (RSRHSSYPAGT):** Use at a final assay concentration of 300 μ M. Make up a 3mM stock. Add 2.5 μ l of stock per assay point.
- 3. Triton X-100:** Make up a 10% (w/v) stock. Add 0.25 μ l of stock per assay point.
- 4. Pim-3, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1–8ng per assay point.
- 5. [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 reaction buffer per assay.
2. Add 2.5 μ l of **(RSRHSSYPAGT)**.
3. Add 0.25 μ l of 10% (w/v) Triton X-100.
4. Add **2.5 μ l (1–8ng) Pim-3, active**.
5. Add 4.75 μ l of dH₂O.
6. Add 10 μ l of diluted [γ -³³P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
9. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat**.
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. 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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PIM3 Sequence Information

<u>Protein</u>	Human Pim-3
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	L32 of recombinant sequence is equivalent to L2 of native human Pim-3
<u>Accession number</u>	GenBank AB114795

Recombinant Pim-3 amino acid sequence:

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1 MSYYHHHHH DYDIPTTENL YFQGAMGIRN SLLSKFGSLA HLCGPGGVDH LPVKILQPAK
61 ADKESFEKAY QVGAVLGSGG FGTVYAGSRI ADGLPVAVKH VVKERVTEWG SLGGATVPLE
121 VLLLRKVGAA GGARGVIRLL DWFERPDPGL LVLERPEPAQ DLDFDITERG ALDEPLARRF
181 FAQVLA AVRH CHSCGVVHRD IKDENLLVDL RSGELKLIDF GSGALLKDTV YTDFDGRTRY
241 SPPEWIRYHR YHGRSATVWS LGVLLYDMVC GDIPFEQDEE ILRGRLLFRR RVSPECQQLI
301 RWCLSLRPSE RPSLDQIAAH PWMLGADGGA PESCDLRLCT LDPDDVASTT SSESSEL
  
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Recombinant Pim-3 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatggg gatccggaat tccctgctct ccaagttcgg ctccctggcg
121 cacctctgcg ggcccggcgg cgtggaccac ctcccgtgta agatcctgca gccagccaag
181 gcggacaagg agagcttcga gaaggcgtac caggtgggcg ccgtgctggg tagcggcggc
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1021 ctcgaccctg atgacgtggc cagcaccagc tccagcagcg agagcttgtg a
  
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