

Certificate of Analysis

PIM1

Pim-1 oncogene

Recombinant Human Active Protein Kinase

HGNC Symbol: PIM1

Synonyms: PIM

Product No.: 0186-0000-1

Lot: 003

Description: Human PIM1, full length, amino acids M₁-K₃₁₃ (as in NCBI/Protein entry NP_002639.1), N-terminal GST-HIS₆ fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: PIM1 Lot 003, was confirmed as PIM1 by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 65,423 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

Storage buffer: 50 mM TRIS-HCL pH 8.0, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol

Storage temperature: -80°C

For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.185 µg/µl

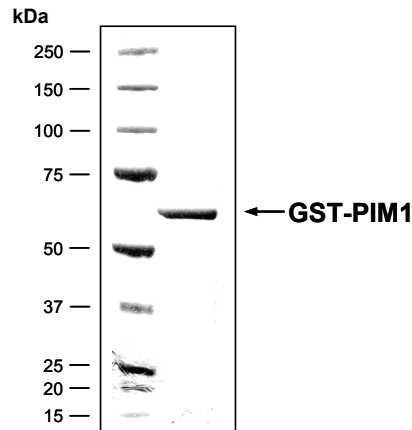
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

Specific kinase activity (P_i transfer): 273 pmol/µg×min

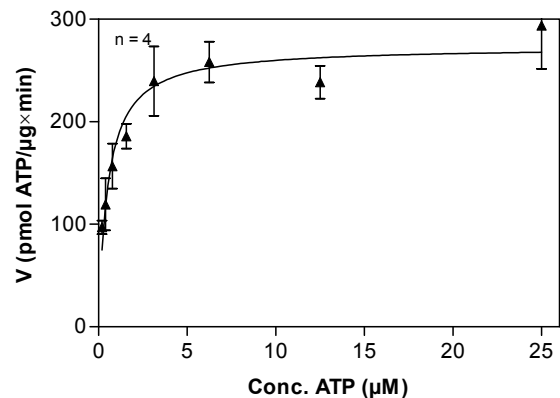
ATP-K_M: 0.5 µM

**PIM1 Lot 003:
Coomassie stain**



2.0 µg GST-PIM1

**PIM1 Lot 003:
Determination of V_{max} and K_M value for ATP**



Determination of K_M value & Specific activity:

• Assay conditions:

60 mM HEPES-NaOH, pH 7.5

3 mM MgCl₂

3 mM MnCl₂

3 µM Na-orthovanadate

1.2 mM DTT

50 µg / ml PEG_{20,000}

ATP (variable)

Substrate: R₁₁-GSK3(14-27)

(R₁₁-SGRARTSSFAEPGGK), 100 µg / ml

PIM1: 0.1 µg / ml

• Filter binding assay

MSPH membrane (Millipore)

Additional assay technology: PIM1 Lot 003

was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega. ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

PIM1

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PIM1 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIQID	KYLKSSKYIA	WPLQGQWQATF	GGGDHPPKSD	PMGHHHHHGG	RRRASVAAGI	240
241	LVPRGSPGLD	GIYARGIQML	LSKINSLAHL	RAAPCNDLHA	TKLAPGKEKE	PLESQYQVGP	300
301	LLGSGGFGSV	YSGIRVSDNL	PVAIKHVEKD	RISDWGELPN	GTRVPMEVVL	LKKVSSGFSG	360
361	VIRLLDWFER	PDSFVLILER	PEPVQDLDFD	ITERGALQEE	LARSFFWQVL	EAVRHCHNCG	420
421	VLHRDIKDEN	ILIDLNRGEL	KLIDFGSGAL	LKDTVYTFDF	GTRVYSPPEW	IRYHRYHGRS	480
481	AAVWSLGILL	YDMVCGDIPF	EHDEEIRGQ	VFFRQRVSSE	CQHLIRWCLA	LRPSDRPTFE	540
541	EIQNHPPMQD	VLLPQETAET	HLHSLSPGPS	K			600

1-218: GST **Red:** HIS6-tag **Pink:** Thrombin cleavage site **blue:** PIM1

PIM1 wt ¹ Amino Acid Sequence							
1	MLLSKINSLA	HLRAAPCNDL	HATKLAPGKE	KEPLESQYQV	GPLLGSGGFG	SVYSGIRVSD	60
61	NLPVAIKHVE	KDRISDWGEL	PNGTRVPMEV	VLLKKVSSGF	SGVIRLLDWF	ERPDSFVLIL	120
121	ERPEPVQDLF	DFITERGALQ	EELARSFFWQ	VLEAVRHCHN	CGVLHRDIKD	ENILIDLNRG	180
181	ELKLIDFGSG	ALLKDTVYTD	FDGTRVYSP	EWIRYHRYHG	RSAAVWSLGI	LLYDMVCGDI	240
241	PFEHDEEIR	GQVFFRQRVS	SECQHLIRWC	LALRPSDRPT	FEEIQNHPPM	QDVLLPQETA	300
301	EIHLSLSPG	PSK					360

blue: PIM1 sequence expressed in fusionprotein

¹NCBI/Protein accession number NP_002639.1