

Certificate of Analysis



PIM3

Pim3 oncogene

Recombinant Human Active Protein Kinase

HGNC Symbol: PIM3

Synonyms: /

Product No.: 0932-0000-1

Lot: 007

Description: Human PIM3, full length, amino acids M₁-L₃₂₆ (as in NCBI/Protein entry NP_001001852.1), N-terminal GST-HIS₆ fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: PIM3 Lot 007, was confirmed as PIM3 by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 64,368 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol

Storage temperature: -80°C

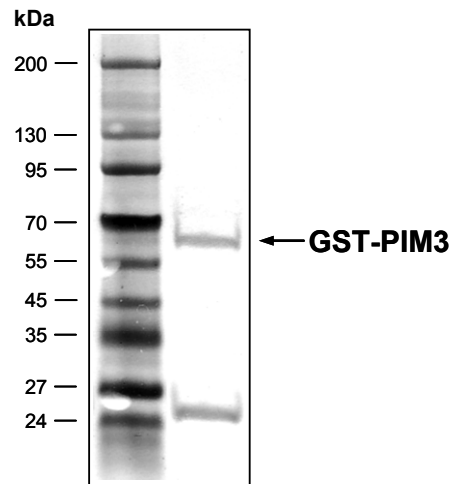
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.157 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

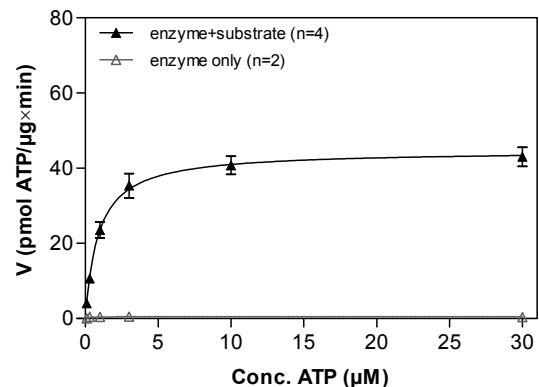
Specific kinase activity (P_i transfer): 45 pmol/µg×min
ATP-K_M: 0.9 µM

**PIM3 Lot 007:
Coomassie stain**



2.0 µg GST-PIM3


**PIM3 Lot 007:
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: GSK3-derived peptide (R11-SGRARTSSFAEPGGK), 80 µg / ml
 - PIM3: 2.0 µg / ml
- Filter binding assay
 - MSFC membrane (Millipore)

Additional assay technology: PIM3 Lot 007

was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from 

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PIM3

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PIM3 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMG HHHHHH HG	RDS LEVL FQG	240
241	PLAMV MLLSK	FGSLAHL CGP	GGVDHL PVKI	LQPAKAD KES	FEKAYQ VGAV	LGSGGF GTVY	300
301	AGSRIAD GLP	VAVKHV VKER	VTEWGS LGGA	TVPLEV VLLR	KVGAAG GARG	VIRLLD WFER	360
361	PDGFL LVLER	PEPAQD LDFD	ITERGAL DEP	LARRFFA QVL	AAVRHCH SCG	VVHRD IKDEN	420
421	LLVDLR SGEL	KLIDFG SGAL	LKDTVY TDFD	GTRVYS PPEW	IRYHRY HGRS	ATVWSL GVLL	480
481	YDMVCG DIPF	EQDEE ILRGR	LLFRRR VSPE	CQQLIR WCLS	LRPSE RPSLD	QIAAHP WMLG	540
541	ADGGAP ESCD	LRLCTL DPDD	VASTTSS SES	L			600

1-218: GST **Red:** HIS6-tag **Green:** 3C cleavage site **blue:** PIM3

PIM3 wt ¹ Amino Acid Sequence							
1	MLLSK FGSLA	HLCGPG VDH	LPVKIL QPAK	ADKES FEKAY	QVGAVL GSGG	FGTVY AGSRI	60
61	ADGLP VAVKH	VVKERV TEWG	SLGGAT VPLE	VVLLRK VGAA	GGARG VIRLL	DWFER PDGFL	120
121	LVLER PEPAQ	DLDFDI TERG	ALDEPL LARRF	FAQVLA AVRH	CHSCG VVHRD	IKDEN LLVDL	180
181	RSGEL KLIDF	GSGALL KDTV	YTDFDG TRVY	SPPEWI RYHR	YHGRS SATVWS	LGVLL YDMVC	240
241	GDIPF EQDEE	ILRGR LLFRR	RVSPEC QQLI	RWCLS LRPSE	RPSLD QIAAH	PWMLG ADGGA	300
301	PESCD LRLCT	LDPDD VASTT	SSSESL				360

blue: PIM3 sequence expressed in fusionprotein

¹NCBI/Protein accession number NP_001001852.1

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