

# Certificate of Analysis



## PIM2

Serine/threonine-protein kinase Pim-2

Recombinant Human Active Protein Kinase

HGNC Symbol: PIM2

Synonyms: n/a

Product No.: 0223-0000-1

Lot: 002

**Description:** Human PIM2, full length, amino acids M<sub>1</sub>-P<sub>311</sub> (as in NCBI/Protein entry NP\_006866.2), N-terminal GST-HIS<sub>6</sub> fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

**Product identity:** PIM2 Lot 002, was confirmed as PIM2 by specific Western Blotting using anti PIM2 antibody

**Theoretical MW**<sub>Fusion Protein</sub>: 64,096 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, 4 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:** 0.216 µg/µl

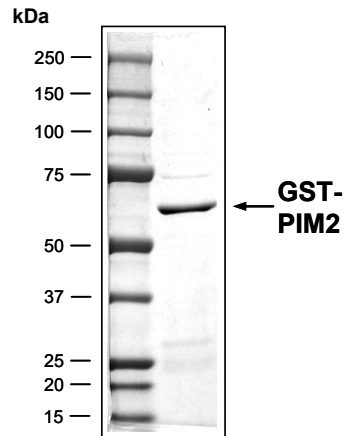
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

### Biochemical Parameters:

Specific kinase activity (P<sub>i</sub> transfer): 35 pmol/µg×min

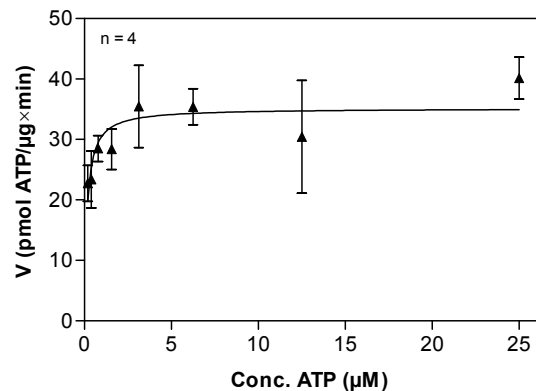
ATP-K<sub>M</sub>: 0.15 µM

### PIM2 Lot 002: Coomassie stain



2.0 µg GST-PIM2

### PIM2 Lot 002: Determination of V<sub>max</sub> and K<sub>M</sub> value for ATP



### Determination of K<sub>M</sub> value & Specific activity:

- Assay conditions:
  - 60 mM HEPES-NaOH, pH 7.5
  - 3 mM MgCl<sub>2</sub>
  - 3 mM MnCl<sub>2</sub>
  - 3 µM Na-orthovanadate
  - 1.2 mM DTT
  - 50 µg / ml PEG<sub>20,000</sub>
  - ATP (variable)
  - Substrate: R<sub>11</sub>-GSK3(14-27) (R<sub>11</sub>-SGRARTSSFAEPGGK), 100 ng/ml
  - PIM2: 100 ng/ml
- Filter binding assay
  - MSFC membrane (Millipore)

### Additional assay technology: PIM2 Lot 002

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



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PIM2 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	KRIEAIQID	KYLKSSKYIA	WPLQGQWQATF	GGGDHPPKSD	PMGHHHHHHG	RRRASVAAGI	240
241	LVPRGSPGLD	GICSRNSPLM	LTKPLQPPA	PPGTPTPPP	GKDREAFAEA	YRLGPLLGKG	300
301	GFGTVFAGHR	LTDRLQVAIK	VIPRNRVLGW	SPLSDSVTCP	LEVALLWKVG	AGGGHPGVIR	360
361	LLDWFETQEG	FMLVLERPLP	AQDLFDYITE	KGPLGEGPSR	CFFGQVVAAI	QHCHSRGVVH	420
421	RDIKDENILI	DLRRGCAKLI	DFGSGALLHD	EPYTDFDGTR	VYSPPEWISR	HQYHALPATV	480
481	WSLGILLYDM	VCRDIPFERD	QEILEAELHF	PAHVSPDCCA	LIRRCLAPKP	SSRPSLEEIL	540
541	LDPWMQTPAE	DVPLNPSKGG	PAPLAWSLLP				600

1-218: GST **Red:** HIS6-tag **Pink:** Thrombin cleavage site **blue:** PIM2

PIM2 wt <sup>1</sup> Amino Acid Sequence							
1	MLTKPLQGPP	APPGTPTPPP	GGKDREAFAEA	EYRLGPLLGK	GFGTVFAGH	RLTDLRQVAI	60
61	KVIPRNRVLG	WSPLSDSVTC	PLEVALLWKV	GAGGGHPGVI	RLDWFETQE	GFMLVLERPL	120
121	PAQDLFDYIT	EKGPLGEGPS	RCFFGQVVAI	IQHCHSRGVV	HRDIKDENIL	IDLRRGCAKL	180
181	IDFGSGALLH	DEPYTDFDGT	RVYSPPEWIS	RHQYHALPAT	VWSLGILLYD	MVCGDIPFER	240
241	DQEILEAELH	FPAHVSPDCC	ALIRRCLAPK	PSSRPSLEEI	LLDPWMQTPA	EDVPLNPSKG	300
301	GPAPLAWSLL	P					360

**blue:** PIM2 sequence expressed in fusionprotein

<sup>1</sup>NCBI/Protein accession number NP\_006866.2