KinaseDetect ApS
http://www.kinasedetect.com

Technical support:
Phone: +45 66 10 66 62
e-mail:support@kinasedetect.com

Certificate of Analysis

Product	**PIM1, active, dephosphorylated** **human recombinant, expressed in E.coli, N-His-fusion protein**
Cat No	PK-032-01
Lot No	010711
Description	Purified and dephosphorylated recombinant human PIM1 expressed in E.coli. Purified by Ni-NTA agarose chromatography, ion exchange chromatography and gel filtration. Suitable for crystallization. Sequence based calculated M.W. 37,450 Approved HUGO gene symbol: PIM1 Synonyms: PIM1 oncogene

Quality

Protein concentration (Bradford with BSA as standard)	1 mg/ml
Purity	> 95% by SDS PAGE
Specific activity (* 1 Unit is defined as 1 picomole phosphate transferred to synthetic peptide (KKRNRTLTV) per min at 30 °C)	3,800,000 Units*/ mg
Protease activity (Twining test)	none

Form	Liquid. In 50 mM Tris-HCl, 500 mM NaCl, 1 mM DTT, pH 7.5
Package size	as requested
Storage condition	-70 ^{O}C
Shipment conditions	dry ice

Material for in vitro research use only. Not for pharmaceutical or drug application. Material does not contain any animal products such as albumin.

AVOID FREEZE/THAW CYCLES

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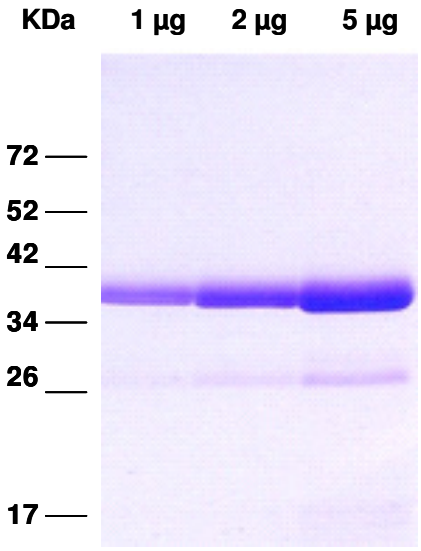
SDS-PAGE analysis



Amino acid sequence information

M17 corresponds to M1 in BC020224

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MGSSHHHHHH SQDPNSMLLS KINSLAHLRA APCNDLHATK LAPGKEKEPL  50
ESQYQVGPLL GSGGFGSVYS GIRVSDNLPV AIKHVEKDRI SDWGELPNGT 100
RVPMEVVLLK KVSSGFSGVI RLLDWFERPD SFVLILERPE PVQDLFDFIT 150
ERGALQEELA RSFFWQVLEA VRHCHNCGVL HRDIKDENIL IDLNRGELKL 200
IDFGSGALLK DTVYTDFDGT RVYSPPEWIR YHRYHGRSAA VWSLGILLYD 250
MVCGDIPFEH DEEIIRGQVF FRQRVSSECQ HLIRWCLALR PSDRPTFEEI 300
QNHPWMQDVL LPQETAEIHL HSLSPGPSK                       329
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In vitro Kinase Assay

Assay components

Assay buffer (AB): 50 mM Tris-HCl, 10 mM DTT, pH 7.5
Enzyme dilution buffer (EB): Assay buffer containing 0,1 % CHAPS
Substrate: PIM-1 peptide, KKRNRTLTV, 400 microM in H2O
Protein kinase: PIM-1, 1 - 5 ng/microliter diluted in EB
Magnesium/ATP Cocktail: 75 mM $MgCl_2$, 500 microM ATP
Diluted [gamma–^{32}P]ATP: Mix 197 microliter Magnesium/ATP cocktail with 3 microliter (30 microCi) [gamma–^{32}P]ATP (3,000 Ci/mmol, e.g. from Hartmann Analytic, Braunschweig, Germany)

Assay procedure

All compounds are pipetted into a microcentrigue tube on ice
1. Add 10 microliter AB
2. Add 10 microliter 400 microM PIM-1 peptide
3. Add 10 microliter PIM-1 (10 - 50 ng/assay)
4. Add 10 microliter of the diluted [gamma–^{32}P]ATP
5. Incubate 10 min at 30 ºC.
6. Stop the reaction by setting samples on ice
7. Remove 10 microliter and spot on P81 paper (let bind to the paper for 30 sec)
8. Immerse the paper in 0.75% phosphoric acid, gently shake on a rotator
9. Wash 3 x with phosphoric acid
10. Wash 1 x with acetone
11. Dry under infrared light
12. Read in scintillation counter or Instant Imager

Human PIM-1 and malignancy

Human PIM-1 has multiple roles in tumorigenesis. It promotes early transformation, cell proliferation and cell survival. In addition it may have a role in angiogenesis and vasculogenesis as a downstream effector of the VEGF-A/Flk1 pathway. PIM-1 expression is correlated with tumor aggressiveness and is a marker for poor prognosis. PIM-1 expression can be predictive of tumour outcome following chemotherapy and surgery and has been correlated with the enhanced metastatic potential of the tumor. For a detailed review refer to Shah et al..

Reference

Shah NS, Pang B, Yeoh KG, Thorn S, Chen CS, Lilly MB, Salto-Tellez M (2008) Potential roles for the PIM1 kinase in human cancer – A molecular and therapeutic appraisal. Eur. J. Cancer. 44, 2144-2151.

Bullok AN, Debreczeni J, Amos AL, Knapp S, Turl BE (2005) Structure and substrate specificity of the Pim-1 kinase. J. Biol. Chem. 280, 41675-41682