

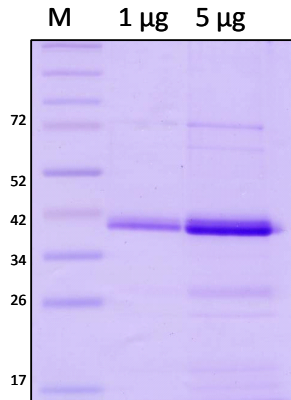
## Certificate of Analysis

<b>Product</b>	<b>PIM1, active</b>	
	<b>human recombinant, expressed in E.coli, N-His-fusion protein</b>	
Cat No	PK-031-01	
Lot No	060710	
<b>Description</b>	Purified recombinant human PIM-1 expressed in E.coli. Suitable for labeling PIM-1 substrates. Features a poly-histidine tag to facilitate removal of PIM-1 from the reaction mixture. Purified by Ni-NTA agarose chromatography. Sequence based calculated M.W. 37,450 Approved HUGO gene symbol: PIM1 Synonyms: PIM1 oncogene	
<b>Quality</b>	Protein concentration (Bradford with BSA as standard)	1 mg/ml
	Purity	> 90% by SDS PAGE
	Specific activity (* 1 Unit is defined as 1 picomole phosphate transferred to synthetic peptide (KKRNRTLTV) per min at 30 °C)	697,500 Units*/ mg
	Protease activity (Twining test)	none
<b>Form</b>	Liquid. In 50 mM Tris-HCl, 500 mM NaCl, 1 mM DTT, pH 8.5	
<b>Package size</b>	20 µg	
<b>Storage condition</b>	-70 °C	
<b>Shipment conditions</b>	dry ice	

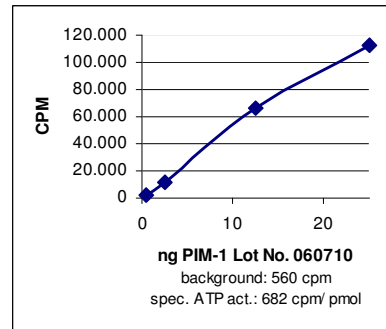
*Material for in vitro research use only. Not for pharmaceutical or drug application. Material does not contain any animal products such as albumin.*

**AVOID FREEZE/THAW CYCLES**

## SDS-PAGE analysis



## Activity determination



## Amino acid sequence information

M17 corresponds to M1 in BC020224

MGSSHHHHH	SQDPNSMLLS	KINSLAHLRA	APCNDLHATK	LAPGKEKEPL	50
ESQYQVGPLL	GSGGFGSVYS	GIRVSDNLPV	AIKHVEKDRI	SDWGELPNGT	100
RVPMEVLLK	KVSSGFSGVI	RLLDWFERPD	SFVLILERPE	PVQDLDFDIT	150
ERGalQEELA	RSFFWQVLEA	VRHCHNCGVL	HRDIKDENIL	IDLNRGELKL	200
IDFGSGALLK	DTVYTDFDGT	RVYSPPEWIR	YHRYHGRSAA	VWSLGILLYD	250
MVCGDIPFEH	DEEIIRGQVF	FRQRVSSECQ	HLIRWCLALR	PSDRPTFEEI	300
QNHPWMQDVL	LPQETAIEIHL	HSLSPGPSK			329

## ***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 H<sub>2</sub>O  
Protein kinase: PIM-1, 1 - 5 ng/microliter diluted in EB  
Magnesium/ATP Cocktail: 75 mM MgCl<sub>2</sub>, 500 microM ATP  
Diluted [ $\gamma$ -<sup>32</sup>P]ATP: Mix 197 microliter Magnesium/ATP cocktail with 3 microliter (30 microCi) [ $\gamma$ -<sup>32</sup>P]ATP (3,000 Ci/mmol, e.g. from Hartmann Analytic, Braunschweig, Germany)

### **Assay procedure**

All compounds are pipetted into a microcentrifuge 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 [ $\gamma$ -<sup>32</sup>P]ATP
5. Incubate 10 min at 30 °C.
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.