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Product Specification

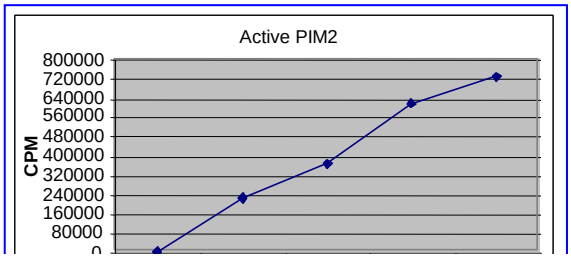
PIM2 active
(Full-length recombinant human PIM-2 was expressed in Sf 9 cells using an N-terminal GST tag

Catalog #: 7730-5
Lot #: _____
Aliquot size: 5 µg protein in 50 µl
Specific activity: 305 nmol/min/mg

Quality Control Analysis
Activity assessment
Pim2 protein (100 ng/µl concentration) was diluted to 20ng/µl with assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM $MgCl_2$, 0.05 mM DTT), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the S6K substrate peptide (CKRRRLASLR) in the following assay condition:

10 µl Diluted Pim2 protein
10 µl S6K substrate peptide (1 mg/ml stock)
5 µl [^{32}P] ATP mixture (250 µM ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [^{32}P] ATP, were incubated at 30° C and the reaction started by the addition of [^{32}P] ATP. After 15 minutes, the reaction was terminated by spotting 20 µl of the reaction mixture onto a phosphocellulose P81 paper. The P81 paper was dried and washed several times in 1% phosphoric acid prior to counting in the presence of scintillation fluid in a scintillation counter. The actual counts, using various dilutions of the enzyme in the assay, are shown in Fig. 1.



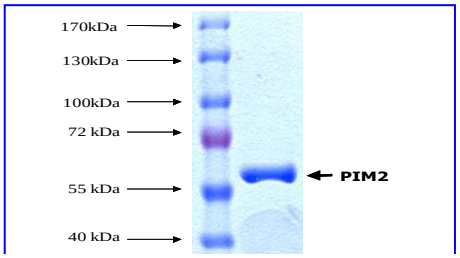


Fig. 1 Pim2 activity assay

Fig. 2 Pim2 protein gel

Purity assessment
2 µg of protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >95% purity of the PIM 2 product, and the band was at ~61 kDa (Fig. 2).
Product Description

Recombinant full-length human PIM 2 containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells. The gene accession number is NM_006875.
This material is sold for research purposes only.

Specific Activity
305 nmol phosphate incorporated into the S6K substrate peptide per minute per mg protein at 30° C for 15 minutes using a final concentration of 50 µM ATP (0.83 µCi/assay).

Formulation
Recombinant proteins in storage buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol).

Storage and Stability
Store product frozen at or below -70° C. Stable for 1 year at -70° C as undiluted stock. Aliquot to avoid repeated thawing and freezing.

Scientific Background
Baytel et al. identified a deduced 334-amino acid sequence of the clone, which showed 90% identity with the mouse Pim2 protein. Like mouse Pim2, the human protein appears to be a serine threonine kinase. Northern blot analysis detected 2 PIM2 transcripts in all tissues tested, but most abundantly in hematopoietic tissues, spleen, thymus, and peripheral blood leukocytes, as well as in testis, small intestine, and colon. It was also highly expressed in human leukemic and lymphoma cell lines and a colorectal adenocarcinoma cell line. The results suggested a role for PIM2 in proliferating cells as well as during meiosis (1). Yan et al investigated potential functions for the pim family of kinases in factor-dependent murine hematopoietic cells and indicate that pim-2 functions similarly to pim-1 as a pro-survival kinase and suggest that BAD is a legitimate PIM-2 substrate (2). Hammerman et al concluded that the transcriptional induction of Pim-2 initiated a novel NF-kappaB activation pathway that regulates cell survival (3).

References
1. Baytel, D.; Shalom, S.; Madgar, I.; Weissenberg, R.; Don, J.: The human Pim-2 proto-oncogene and its testicular expression. *Biochim. Biophys. Acta* 1442: 274-285, 1998.
2. Yan B, Zemskova M, Holder S, Chin V, Kraft A, Koskinen PJ, Lilly M. The PIM-2 kinase phosphorylates BAD on serine 112 and reverses BAD-induced cell death. J Biol Chem. 2003 Nov 14;278(46):45358-67. Epub 2003 Sep 3.
3. Hammerman PS, Fox CJ, Cinalli RM, Xu A, Wagner JD, Lindsten T, Thompson CB. Lymphocyte transformation by Pim-2 is dependent on nuclear factor-kappaB activation. Cancer Res. 2004 Nov 15;64(22):8341-8.

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