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Product Specification

Pim 1 active
(Full-length recombinant protein expressed in Sf 9 cells)

Catalog #	7742-5
Lot #	----------
Aliquot size:	5 µg protein in 50 µl
Specific activity:	316 nmol/min/mg

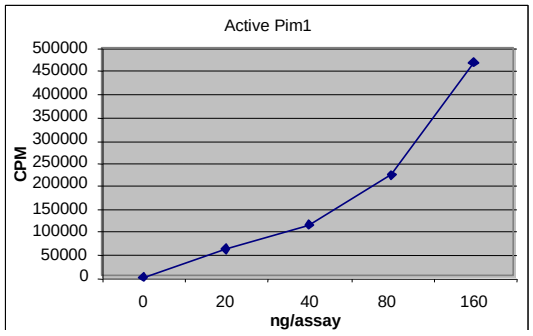
Quality Control Analysis

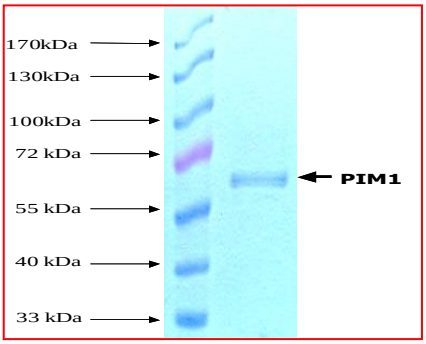
Activity assessment

Pim1 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 Pim1 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.





Purity assessment

1 µg of Pim1 protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the Pim1 product, and the band was at ~62 kDa (Fig. 2)

Product Description

Recombinant full length human Pim1 containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells. The gene accession number is NM_002648.
This material is sold for research purposes only.

Specific Activity
316 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
The proto-oncogene Pim1 belongs to a family of serine/threonine protein kinases that are highly conserved through evolution in multicellular organisms. Originally identified from Moloney murine leukemia virus induced T-cell lymphomas in mice, Pim1 is involved in the control of cytokine-mediated cell proliferation, differentiation and survival of lymphoid and myeloid cells as well as others. Expression of Pim1 can be stimulated by a variety of growth factors and is regulated at four different levels: transcriptional, post-transcriptional, translational and post-translational. Accumulating data support that the expression of Pim1 is mediated through activation of the JAK/STAT pathway. Some of the substrates of Pim1 include p21 Cip1, nuclear mitotic appartus protein, PTP-U2S and Socs-1. Recently, Pim1 has been shown to enhance the activities of p100, c-Myb and Cdc 25a and in part this might explain reported effects of Pim1 on mitogenesis. Pim1 interacts with c-Myb via the DNA binding domain and regulates its transcriptional activity.

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