

Product: PIM1, Active

Catalog #: 02-2036

Amount: 5 µg

Product Description

Recombinant full-length human PIM1 was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is [NM_002648](#).

Gene Aliases

PIM

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

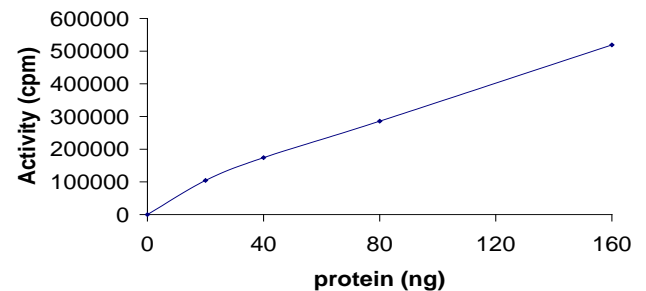
Scientific Background

PIM1 is a proto-oncogene that 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 (1). 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 (2). Expression of PIM1 is mediated through activation of the JAK/STAT pathway.

References

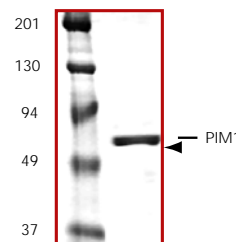
1. Meeker, TC. et al: Cloning and characterization of the human PIM-1 gene: a putative oncogene related to the protein kinases. *J Cell Biochem.* 1987 Oct;35(2):105-12.
2. Friedmann, M. et al: Characterization of the proto-oncogene pim-1: kinase activity and substrate recognition sequence. *Arch Biochem Biophys.* 1992 Nov 1;298(2):594-601.

Specific Activity



The specific activity of PIM1 was determined to be **316 nmol/min/mg** as per activity assay protocol.

Purity



The purity was determined to be **>90%** by densitometry. Approx. MW **62kDa**.

PIM1, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number **02-2036**
 Quantity **5µg**
 Specific Activity **316 nmol/min/mg**
 Specific Lot Number **A354-1**

Purity **>90%**
 Format **5µg in 50µl**
 Concentration **0.1µg/µl**
 Stability **1yr At -70°C from date of shipment**
 Storage & Shipping **Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.**

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: 02-2036)

Active PIM1 (0.1µg/µl) diluted with Kinase Dilution Buffer (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active PIM1 for optimal results).

Kinase Dilution Buffer, pH 7.2 (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50 ng/µl BSA solution.

Kinase Assay Buffer I, pH 7.2 (Catalog #: K01-09)

Buffer components: 25mM MOPS, 12.5mM β-glycerol-phosphate, 25mM , 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³²P]-ATP Assay Cocktail

Prepare 250µM [³²P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³²P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer (Catalog #: K01-09). Store 1ml aliquots at -20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer (Catalog #: K01-09). Store 200µl aliquots at -20°C.

Substrate (Cat#: S05-58)

S6K synthetic peptide substrate (CKRRRLASLR) diluted in distilled to a final concentration of 1mg/ml.

Assay Protocol

- Step 1. Thaw [³²P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2. Thaw the Active PIM1, Kinase Assay Buffer, Substrate and Enzyme Dilution Buffer on ice.
- Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:
 - Component 1. 10µl of diluted Active PIM1 (Catalog #02-2036)
 - Component 2. 10µl of 1mg/ml stock solution of substrate
- Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H₂O.
- Step 5. Initiate the reaction by the addition of 5µl [³²P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H₂O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [³²P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5µl [³²P]-ATP / pmoles of ATP (in 5µl of a 250µM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ³²P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*(Reaction Volume) / (Spot Volume)]

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