Recombinant Human Active PIM1 Protein

Catalog Number: 4588-KS-010
Lot Number: 1762435

SPECIFICATIONS AND USE

Source	Recombinant human PIM1 was expressed by baculovirus in *Sf*9 insect cells using an N-terminal GST tag. The gene accession number is NM_002648.
Molecular Mass	The approximate molecular weight is 62 kDa.
Purity	The purity was determined to be > 90% by densitometry.
Formulation	Stored in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM Glutathione, 0.1 mM EDTA, 0.25 mM DTT, 0.1 mM PMSF, and 25% Glycerol.
Size	10 µg
Concentration	0.1 µg/µL
Activity	The specific activity of PIM1 was determined to be 30 nmol/min/mg using a S6K synthetic peptide substrate (KRRRLASLR).
Storage	This product is stable at ≤ -70 °C for up to one year from the date of receipt. For optimal storage, aliquot into smaller quantities after centrifugation and store at recommended temperature. **Avoid repeated freeze-thaw cycles.**

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.

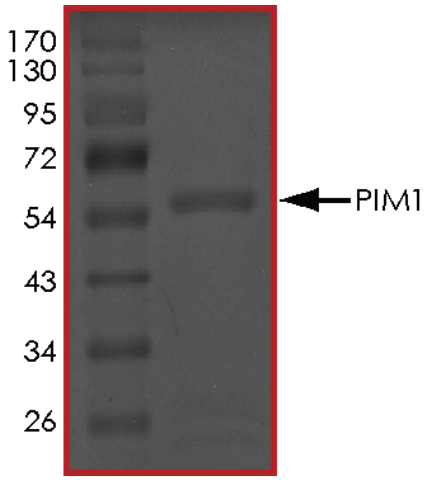

Figure 1: The approximate molecular weight is 62 kDa and the purity is > 90%.

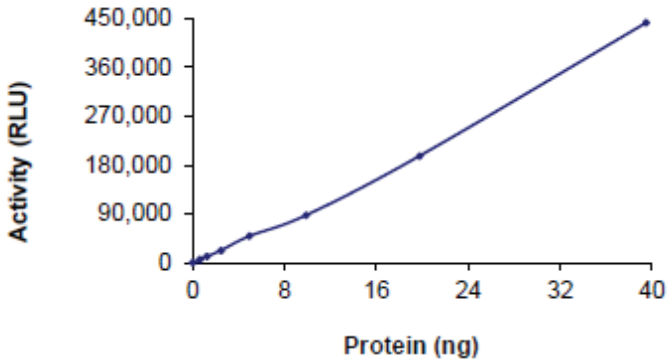

Figure 2: The specific activity of PIM1 was determined to be 30 nmol /min/mg as per Activity Assay Protocol and was equivalent to 280 nmol/min/mg as per radiometric assay.

REFERENCES

1. Meeker, TC. *et al.* (1987) J Cell Biochem **35**:105.
2. Friedmann, M. *et al.* (1992) Arch Biochem Biophys. **298**:594.

Global bio-techne.com info@bio-techne.com TEL +1 612 379 2956 **USA** TEL 800 343 7475 **Canada** TEL 855 668 8722
Europe | Middle East | Africa TEL +44 (0)1235 529449 **China** TEL +86 (21) 52380373

ACTIVITY ASSAY PROTOCOL

Solutions Required

Active Kinase - Active PIM1 (0.1 µg/µL) diluted with Kinase Dilution Buffer III to the concentrations indicated in Figure 2.
Note: *These are suggested working dilutions. Optimal dilutions should be determined by each laboratory for each application.*

Kinase Assay Buffer III (5X) - 200 mM Tris-HCl, pH 7.4, 100 mM $MgCl_2$, and 0.5 mg/mL BSA. Add fresh DTT prior to use to a final concentration of 250 µM.

Kinase Dilution Buffer IX (1X) - Kinase Assay Buffer III diluted at a 1:4 ratio (5X dilution) with cold distilled water. Add fresh DTT prior to use to a final concentration of 50 µM.

ADP-Glo™ Kinase Assay Kit - 10 mM ATP Solution, 10 mM ADP Solution, ADP-Glo™ Reagent, Kinase Detection Reagent.

Substrate - S6K synthetic peptide substrate (KRRRLASLR) diluted in distilled water to a final concentration of 1 mg/mL.

ASSAY PROCEDURE

The PIM1 assay is performed using the ADP-Glo Kinase Assay Kit which quantifies the amount of ADP produced by the PIM1 reaction. The ADP-Glo Reagent is added to terminate the kinase reaction and to deplete the remaining ATP, and then the Kinase Detection Reagent is added to convert ADP to ATP and to measure the newly synthesized ATP using luciferase/luciferin reaction.

1. Thaw the Active PIM1, Kinase Assay Buffer III (5X), and Substrate on ice. Prepare a 15 µL enzyme dilution using Kinase Dilution Buffer IX (1X), at the desired concentration, in a pre-chilled 96-well plate.
2. Prepare a substrate/ATP mixture as follows (25 µM ATP example):

Reaction Component	Amount
10 mM ATP Solution	1 µL
Kinase Assay Buffer III (5X)	79 µL
Substrate at 1 mg/mL	80 µL

3. Transfer the following reaction components prepared in step 1 and 2 to a 384-well opaque plate bringing the reaction volume up to 5 µL:

Component 1	3 µL of diluted Active PIM1.
Component 2	2 µL of Substrate/ATP mix as prepared in the table above. This initiates the reaction.

4. Set up the blank control as outlined in step 2, excluding the addition of the kinase. Replace the kinase with an equal volume of Kinase Dilution Buffer IX (1X).
5. Incubate at ambient temperature for 40 minutes.
6. After the 40-minute incubation period, terminate the reaction and deplete the remaining ATP by adding 5 µL of ADP-Glo Reagent. Spin down and shake the 384-well plate. Then incubate the reaction mixture for another 40 minutes at ambient temperature.
7. Add 10 µL of the Kinase Detection Reagent to the 384-well plate and incubate the reaction mixture for another 30 minutes at ambient temperature.
8. Read the 384-well reaction plate using the Luminescence Module Protocol on a GloMax®-Multi Microplate Multimode Reader.
9. Determine the corrected activity (RLU) by removing the blank control value (step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of Specific Activity of ADP (RLU/pmol)

From ADP standard curve, determine RLU/pmol of ADP

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

Corrected RLU from reaction / [(SA of ADP in RLU/pmol) * (Reaction time in min) * (Enzyme amount in µg or mg)]