Recombinant Human Active PIM1

Certificate of Analysis

Catalog Number: 4588-KS
Lot Number: 1478416

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 (see Figure 1 below).
Purity	♦ The purity was determined to be > 90% by densitometry (see Figure 1 below).
Formulation	♦ Supplied in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.25 mM DTT, 10 mM glutathione, 0.1 mM EDTA, 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 270 nmol/min/mg using a synthetic peptide substrate (see Activity Assay Protocol).
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.**

PIM1

PIM1 is a proto-oncogene that belongs to a family of Ser/Thr 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, T.C. *et al*. (1987) J. Cell Biochem. **35**:105.
2. Friedmann, M. *et al*. (1992) Arch. Biochem. Biophys. **298**:594.

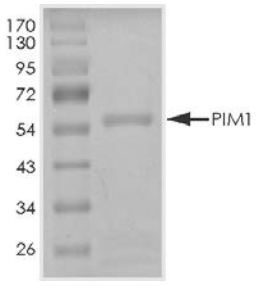

Figure 1: The approximate molecular weight is 62 kDa and the purity is > 90%.

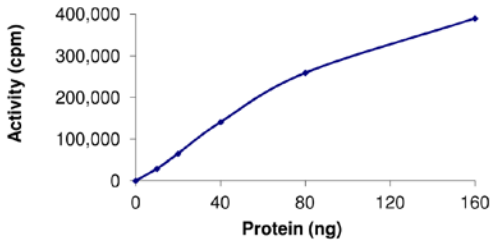

Figure 2: The specific activity of this lot of PIM1 was determined to be 270 nmol/min/mg as per the Activity Assay Protocol (on reverse).

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Activity Assay Protocol

Solutions Required

- **Active Kinase** - Active PIM1 (0.1 μg/μL) diluted with Kinase Dilution Buffer. **Note:** *These are suggested working dilutions. Optimal dilutions should be determined by each laboratory for each application.*
- **Kinase Assay Buffer I, pH 7.2** - 25 mM MOPS, 12.5 mM β-glycerolphosphate, 25 mM $MgCl_2$, 5 mM EGTA, 2 mM EDTA. Add 0.25 mM DTT to the Kinase Assay Buffer prior to use.
- **Kinase Dilution Buffer, pH 7.2** - Kinase Assay Buffer I diluted 5-fold with a 50 ng/μL BSA solution.
- **10 mM ATP Stock Solution** - Prepare the ATP Stock Solution by dissolving 55 mg of ATP in 10 mL of Kinase Assay Buffer I. Store 200 μL aliquots at ≤ -20° C.
- **[^{33}P]-ATP Assay Cocktail** - Prepare 250 μM [^{33}P]-ATP Assay Cocktail in a designated radioactive work area by combining 150 μL of 10 mM ATP Stock Solution, 100 μL of [^{33}P]-ATP (1 mCi/100 μL), and 5.75 mL of Kinase Assay Buffer I. Store 1 mL aliquots at ≤ -20° C.
- **Substrate** - S6K synthetic peptide substrate (KRRRLASLR) diluted in distilled or deionized water to a final concentration of 1 mg/mL.

Assay Procedure

1. Thaw the [^{33}P]-ATP Assay Cocktail in a shielded container in a designated radioactive work area.
2. Thaw the Active PIM1, Kinase Assay Buffer I, Substrate, and Kinase Dilution Buffer on ice.
3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 μL.

Reaction Component	Amount
Diluted Active PIM1	10 μL
Substrate (1 mg/mL; on ice)	5 μL

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 or deionized water.
5. Initiate the reaction with the addition of 5 μL [^{33}P]-ATP Assay Cocktail, bringing the final volume up to 25 μL. Incubate the mixture in a water bath at 30° C for 15 minutes.
6. After the 15 minute incubation, terminate the reaction by spotting 20 μL of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (add 10 mL of phosphoric acid to 990 mL of distilled or deionized water) with constant gentle stirring. It is recommended that the strips be washed a total of three times for approximately 10 minutes each.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by subtracting the blank control value (see step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [^{33}P]-ATP Specific Activity (SA) (cpm/pmol)

Specific Activity (SA) = cpm for 5 μL [^{33}P]-ATP/pmole of ATP (in 5 μL of a 250 μM ATP stock solution, *i.e*. 1250 pmoles)

Calculation of Kinase Specific Activity (SA) (pmol/minutes/μg or nmol/minutes/mg)

Corrected cpm from reaction / [(SA of ^{33}P-ATP in cpm/pmol) x (Reaction time in minutes) x (Enzyme amount in μg or mg)] x [(Reaction volume) / (Spot Volume)]

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