

# Pim-1 Kinase

☑ 5 µg



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TECHNOLOGY®

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This product is for *in vitro* research use only and is not intended for use in humans or animals.

**Description:** Purified recombinant full length human Pim-1 kinase, supplied as a GST fusion protein.

**Background:** Pim proteins (Pim-1, Pim-2 and Pim-3) are oncogene-encoded serine/threonine kinases (1). Pim-1, a serine/threonine kinase highly expressed in hematopoietic cells, plays a critical role in the transduction of mitogenic signals and is rapidly induced by a variety of growth factors and cytokines (1-4). Pim-1 cooperates with c-Myc in lymphoid cell transformation and protects from growth factor withdrawal and genotoxic stress-induced apoptosis (5,6). Pim-1 also enhances the transcriptional activity of c-Myb through direct phosphorylation within the c-Myb DNA binding domain as well as phosphorylation of the transcriptional coactivator p100 (7,8). Hypermutations of the Pim-1 gene are found in B-cell diffuse large cell lymphomas (9). Phosphorylation of Pim-1 at Tyr 218 by Etk occurs following IL-6 stimulation and is correlated with an increase in Pim-1 activity (10). Various substrates have been identified for Pims. Among them, BAD has been shown to be phosphorylated by both Pim-1 and Pim-2 at Ser112 and this phosphorylation reverses BAD-induced cell apoptosis (11,12).

**Source/Purification:** The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full length human Pim-1 (Met1-Lys313) (GenBank Accession No. NM\_002648) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

**Quality Control:** The theoretical molecular weight of the GST-Pim-1 fusion protein is 65 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Silver stain and Western blot [Fig.1]. Pim-1 kinase activity was determined using a radiometric assay [Fig.2]. A kinase dose dependency assay was performed to measure Pim-1 activity using HTScan™ Pim-1 Kinase Assay Kit #7573 [Fig.3].

#### Background References:

- (1) Mikkers, H. et al. (2004) *Mol. Cell.Biol.* 24, 6104–6115.
- (2) Selten, G. et al. (1986) *Cell* 46, 603–611.
- (3) Meeker, T.C. et al. (1987) *J. Cell. Biochem.* 35, 105–112.
- (4) Dautry, F. et al. (1988) *J. Biol. Chem.* 263, 17615–17620.
- (5) Moroy, T. et al. (1993) *Proc. Natl. Acad. Sci. USA* 90, 10734–10738.
- (6) Lilly, M. and Kraft, A. (1997) *Cancer Res.* 57, 5348–5355.
- (7) Levenson, J.D. et al. (1998) *Mol. Cell.* 2, 417–425.
- (8) Winn, L.M. et al. (2003) *Cell Cycle* 2, 258–262.
- (9) Pasqualucci, L. et al. (2001) *Nature* 412, 341–346.
- (10) Kim, O. et al. (2004) *Oncogene* 23, 1838–1844.
- (11) Aho, T. L. et al. (2004) *FEBS Lett.* 571, 43–49.
- (12) Yan, B. et al. (2003) *J. Biol. Chem.* 278, 45358–45367

**Storage:** Enzyme is supplied in 50 mM Tris-HCl, pH 8.0; 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol. Store at -80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

#### Companion Products:

HTScan™ Pim-1 Kinase Assay Kit #7573

Phospho-Bad (Ser112) Antibody #9291

Bad (Ser112) Biotinylated Peptide #1342

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Staurosporine #9953

Serine/Threonine Kinase Substrate Screening Kit #7400

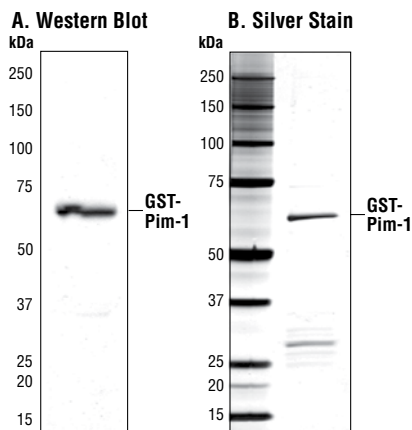


Figure 1. The purity of the GST-Pim-1 fusion protein was analyzed using SDS/PAGE followed by anti-Pim-1 Western blot (A) or Silver stain (B).

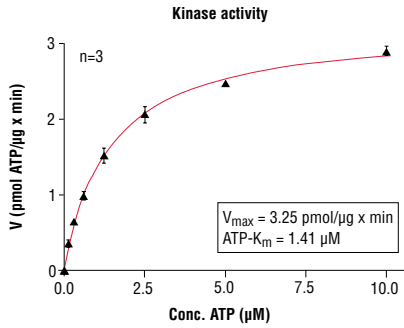


Figure 2. Pim-1 kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM MgCl<sub>2</sub>, 3 mM MnCl<sub>2</sub>, 3 µM Na-orthovanadate, 1.2 mM DTT, ATP (variable), 2.5 µg/50 µl PEG20,000, Substrate: Histone H2B, 5 µg/50 µl and 200 ng/50 µl Recombinant Pim-1.

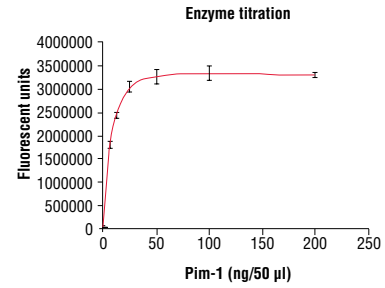


Figure 3. Dose dependence curve of Pim-1 kinase activity: DELFIA® data generated using Phospho-Bad (Ser112) Antibody #9291 to detect phosphorylation of substrate peptide (#1342) by Pim-1 kinase. In a 50 µl reaction, increasing amounts of Pim-1 and 1.5 µM substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

## Protocol for Pim-1 Kinase Assay

**\*IMPORTANT:** Use of an automated microplate washer as well as centrifugation of plates when appropriate, greatly improves reproducibility.

### Kinase

**Note:** Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

### A Additional Solutions and Reagents (Not included)

- 1. Wash Buffer:** 1X PBS, 0.05% Tween-20 (PBS/T)
- Bovine Serum Albumin (BSA)
- 3. Stop Buffer:** 50 mM EDTA pH 8
- Phospho-Bad (Ser112) Antibody #9291
- Kinase Buffer (10X) #9802
- ATP (10 mM) #9804
- Bad (Ser112) Biotinylated Peptide #1342
- DELFI<sup>®</sup> Europium-labeled Anti-rabbit antibody (PerkinElmer Life Sciences #AD0105)
- DELFI<sup>®</sup> Enhancement Solution (PerkinElmer Life Sciences #1244-105)
- DELFI<sup>®</sup> Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

DELFI<sup>®</sup> is a registered trademark of PerkinElmer Life Sciences

### B Suggested Protocol for 100 Assays

- Add 100  $\mu$ l 10 mM ATP to 1.25 ml 6  $\mu$ M substrate peptide. Dilute the mixture with dH<sub>2</sub>O to 2.5 ml to make 2X ATP/substrate cocktail ([ATP]=400  $\mu$ M, [substrate] = 3  $\mu$ M).
- Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
- Microcentrifuge briefly at 4°C to bring liquid to the bottom of the vial. Return immediately to ice.**
- Add 1 ml 10X kinase buffer [250 mM Tris-HCl pH 7.5, 100 mM MgCl<sub>2</sub>, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 50 mM  $\beta$ -glycerophosphate, 20 mM dithiothreitol (DTT)] to 1.5 ml dH<sub>2</sub>O to make 2.5 ml 4X reaction buffer.
- Dilute enzyme in 1.25 ml of 4X reaction buffer to make 4X reaction cocktail ([enzyme]=4.0 ng/ $\mu$ l in 4X reaction cocktail).
- Add 12.5  $\mu$ l of the 4X reaction cocktail to 12.5  $\mu$ l/well of prediluted compound of interest (usually around 10  $\mu$ M) and incubate for 5 minutes at room temperature.
- Add 25  $\mu$ l of 2X ATP/substrate cocktail to 25  $\mu$ l/well preincubated reaction cocktail/compound.

### Final Assay Conditions for a 50 $\mu$ l Reaction

25 mM Tris-HCl (pH 7.5)  
 10 mM MgCl<sub>2</sub>  
 5 mM  $\beta$ -glycerophosphate  
 0.1 mM Na<sub>3</sub>VO<sub>4</sub>  
 2 mM DTT  
 200  $\mu$ M ATP  
 1.5  $\mu$ M peptide  
 50 ng Pim-1 Kinase

- Incubate reaction plate at room temperature for 30 minutes.
- Add 50  $\mu$ l/well Stop Buffer (50 mM EDTA, pH 8) to stop the reaction.
- Transfer 25  $\mu$ l of each reaction to a 96-well streptavidin-coated plate containing 75  $\mu$ l dH<sub>2</sub>O/well and incubate at room temperature for 60 minutes.
- \*Wash three times with 200  $\mu$ l/well PBS/T.
- Dilute primary antibody in PBS/T with 1% BSA. Add 100  $\mu$ l/well primary antibody.  
**Please note:** This protocol was validated using a Bad (Ser112) Biotinylated Peptide and Phospho-Bad (Ser112) Antibody diluted 1:1000 (see additional reagents). Primary antibody chosen should be specific to the substrate used.
- Incubate at 37°C for 120 minutes.
- \*Wash three times with 200  $\mu$ l/well PBS/T.
- Dilute Europium labeled secondary antibody 1:1000 in PBS/T with 1% BSA. Add 100  $\mu$ l/well diluted antibody.
- Incubate at room temperature for 30 minutes.
- \*Wash five times with 200  $\mu$ l/well PBS/T.
- Add 100  $\mu$ l/well DELFI<sup>®</sup> Enhancement Solution.
- Incubate at room temperature for 5 minutes.
- Detect 615 nm fluorescence emission with appropriate Time-Resolved Plate Reader.

Please contact Cell Signaling Technology for HTS-ready antibodies (PBS formulated and carrier-free), and detailed peptide substrate sequence information.  
 Email: drugdiscovery@cellsignal.com