Store at -80°C

#7576

HTScan® Pim-2 Kinase Assay Kit

☑ 100 assays
(96 Well Format)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 09/05/07

Products Included	Products #	Kit Quantity
Phospho-Bad (Ser112) Antibody	9291	30 µl
Kinase Buffer (10X)	9802	15 ml
ATP (10 mM)	9804	1 ml
Bad (Ser112) Biotinylated Peptide	1342	1.25 ml
Pim-2 Kinase (recombinant, human)	7575	5 µg

Description: The kit provides a means of performing kinase activity assays with recombinant human Pim-2 kinase. It includes active Pim-2 kinase (supplied as a GST fusion protein), a biotinylated peptide substrate and a phospho-serine/threonine antibody for detection of the phosphorylated form of the substrate peptide.

Peptide Core Sequence: RS*RHS*S*Y

Molecular Weights: Peptide substrate, Biotin-peptide: 1,700 Daltons. GST-Pim-2 Kinase : 64 kDa.

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full length human Pim-2 (Met1-Pro311) (GenBank Accession No. XM_010208) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

Quality Control: The substrate peptide was selected using our Serine/Threonine Kinase Substrate Screening Kit #7400. Phospho-Bad (Ser112) Antibody #9291 was used for detection. The quality of the biotinylated peptide was evaluated by reverse-phase HPLC and by mass spectrometry.

Purified Pim-2 kinase was quality controlled for purity by SDS-PAGE followed by Coomassie stain and Western blot. The specific activity of the Pim-2 kinase was determined using a radiometric assay [Fig.1]. Time course [Fig.2], kinase dose dependency [Fig.3] and substrate dose-dependency [Fig.4] assays were performed to verify Pim-2 activity using the Pim-2 substrate peptide provided in this kit. Pim-2 sensitivity to the inhibitor staurosporine was measured using the Pim-2 substrate peptide provided in this kit [Fig.5].

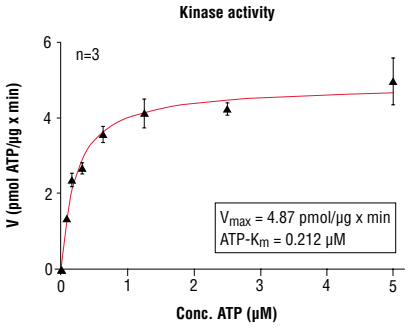

Figure 1. Pim-2 kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM MnCl2, 3 µM Na-orthovanadate, 1.2 mM DTT, ATP (variable), 2.5 µg/50 µl PEG20,000, Substrate: R11-S6-peptide5 µg/50 µl, recombinant Pim-2: 200 ng/50 µl.

Storage: Antibodies are supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Do not aliquot the antibodies. Peptides are supplied at 6 µM in 0.001% DMSO. Enzymes are supplied in 50 mM Tris-HCL (pH 8.0), 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione and 20% glycerol. Store at –80°C.

Keep enzymes on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:
Serine/Threonine Kinase Substrate Screening Kit #7400

Pim-2 Kinase #7575

Phospho-Bad (Ser112) Antibody #9291

Bad (Ser112) Biotinylated Peptide #1342

Staurosporine #9953

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

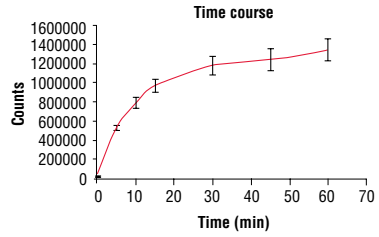

Figure 2. Time course of Pim-2 kinase activity: DELFIA® data generated using Phospho-Bad (Ser112) Antibody #9291 to detect phosphorylation of Pim-2 substrate peptide (#1342) by Pim-2 kinase. In a 50 µl reaction, 50 ng Pim-2 and 1.5 µM substrate peptide were used per reaction. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

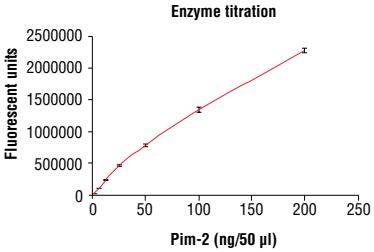

Figure 3. Dose dependence curve of Pim-2 kinase activity: DELFIA® data generated using Phospho-Bad (Ser112) Antibody #9291 to detect phosphorylation of substrate peptide (#1342) by Pim-2 kinase. In a 50 µl reaction, increasing amounts of Pim-2 and 1.5 µM substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

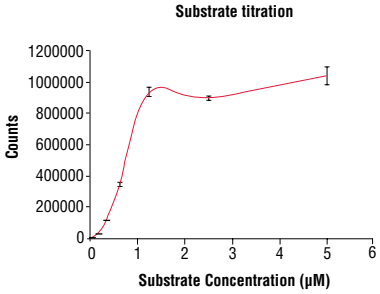

Figure 4. Peptide concentration dependence of Pim-2 kinase activity: DELFIA® data generated using Phospho-Bad (Ser112) Antibody #9291 to detect phosphorylation of substrate peptide (#1342) by Pim-2 kinase. In a 50 µl reaction, 50 ng of Pim-2 and increasing concentrations of substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

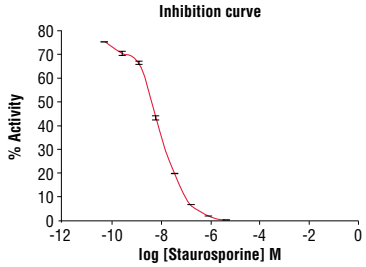

Figure 5. Staurosporine inhibition of Pim-2 kinase activity: DELFIA® data generated using Phospho-Bad (Ser112) Antibody #9291 to detect phosphorylation of Pim-2 substrate peptide (#1342) by Pim-2 kinase. In a 50 µl reaction, 50 ng Pim-2, 1.5 µM substrate peptide, 20 µM ATP and increasing amounts of staurosporine were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

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).

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) Leverson, 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.

Protocol for HTScan® Pim-2 Kinase Assay Kit

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)
2. Bovine Serum Albumin (BSA)
3. **Stop Buffer:** 50 mM EDTA pH 8

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B Suggested Protocol for 100 Assays

1. Add 100 µl 10 mM ATP to 1.25 ml 6 µM substrate peptide. Dilute the mixture with dH_20 to 2.5 ml to make 2X ATP/substrate cocktail ([ATP]=400 µM, [substrate] = 3 µm).
2. Transfer enzyme from –80°C to ice. Allow enzyme to thaw on ice.
3. **Microcentrifuge briefly at 4°C to bring liquid to the bottom of the vial. Return immediately to ice.**
4. Add 1 ml 10X kinase buffer [1 ml 10X Kinase Buffer 250 mM Tris-HCl pH 7.5, 100 mM $MgCl_2$, 1 mM Na_3VO_4, 50 mM β-glycerophosphate, 20 mM dithiothreitol (DTT)] to 1.5 ml dH_20 to make 2.5 ml 4X reaction buffer.
5. Transfer 1.2 ml of 4X Reaction buffer to each enzyme tube to make 4X reaction cocktail ([enzyme]) = 4 ng/µl in 4X reaction cocktail).
6. Add 12.5 µl of the 4X reaction cocktail to 12.5 µl/well of prediluted compound of interest (usually around 10 µM) and incubate for 5 minutes at room temperature.
7. Add 25 µl of 2X ATP/substrate cocktail to 25 µl/well preincubated reaction cocktail/compound.

Final Assay Conditions for a 50 µl Reaction

25 mM Tris-HCl (pH 7.5)
10 mM $MgCl_2$
5 mM β-glycerophosphate
0.1 mM Na_3VO_4
2 mM DTT
200 µM ATP
1.5 µM peptide
50 ng Pim-2 Kinase

8. Incubate reaction plate at room temperature for 30 minutes.
9. Add 50 µl/well Stop Buffer (50 mM EDTA, pH 8) to stop the reaction.
10. Transfer 25 µl of each reaction to a 96-well streptavidin-coated plate containing 75 µl dH_2O/well and incubate at room temperature for 60 minutes.
11. *Wash three times with 200 µl/well PBS/T.
12. Dilute primary antibody, Phospho-Bad (Ser112) Antibody #9291, 1:1000 in PBS/T with 1% BSA. Add 100 µl/well primary antibody.
13. Incubate at room temperature for 120 minutes.
14. *Wash three times with 200 µl/well PBS/T.
15. For DELFIA® or Colorimetric ELISA detection methods please use the following protocols.

DELFIA® Assay

1. Prepare appropriate dilution of Europium labeled secondary antibody in PBS/T with 1% BSA (1:500 dilution for anti-mouse IgG or 1:1000 for anti-rabbit IgG).
2. Add 100 µl/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. *Wash five times with 200 µl/well PBS/T.
5. Add 100 µl/well DELFIA® Enhancement Solution.
6. Incubate at room temperature for 5 minutes.
7. Read plate using a Time Resolved Fluorescent plate reader using the following settings;
   a. Excitation Filter: 340 nm
   b. Emission Filter: 615 nm
   c. Delay++: 400 µs

++ Delay time is the delay from the excitation pulse to the beginning of the measurement.

Companion Products for DELFIA®

DELFIA® Europium-labeled Anti-mouse IgG (PerkinElmer Life Sciences #AD0124)
DELFIA® Europium-labeled Anti-rabbit IgG (PerkinElmer Life Sciences #AD0105)
DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

Colorimetric ELISA Assay

1. Prepare appropriate dilution of HRP labeled secondary antibody in PBS/T with 1% BSA (1:500 dilution for anti-mouse IgG or 1:1000 for anti-rabbit IgG).
2. Add 100 µl/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. *Wash five times with 200 µl/well PBS/T.
5. Add 100 µl/well TMB substrate.
6. Incubate at room temperature for 15 minutes.
7. Add 100 µl/well of stop solution.
8. Mix well.
9. Read the absorbance at 450 nm with a microtiter plate reader.

Companion Products For Colorimetric ELISA Assay

Anti-mouse IgG, HRP Linked Antibody #7076
Anti-rabbit IgG, HRP Linked Antibody #7074
TMB Solution #7004
Stop Solution #7002

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

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