Store at -80°C

#7907

HTScan® Abl1 T315I Kinase Assay Kit

☑ 100 Assays
(96 Well Format)

Cell Signaling TECHNOLOGY®

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Products Included	Product #	Kit Quantity
Phospho-Tyrosine Mouse mAb (P-Tyr-100)	9411	30 µl
HTScan® Tyrosine Kinase Buffer (4X)	9805	15 ml
ATP (10 mM)	9804	1 ml
DTT (1000x, 1.25M)		80 µl
Signal Transduction Protein (Tyr160) Biotinylated Peptide	1366	1.25 ml
Abl1 T315I Kinase	7906	2 X 5 µg

Description: The kit provides a means of performing kinase activity assays with recombinant human Abl1 T315I kinase. It includes active Abl1 T315I kinase (supplied as a GST fusion protein), a biotinylated peptide substrate and a phospho-tyrosine monoclonal antibody for detection of the phosphorylated form of the substrate peptide.

Peptide Core Sequence: GIY*DV

Molecular Weights: Peptide substrate, Biotin-Signal Transductin Protein (Tyr160): 1830 Daltons. GST-Abl1 T315I Kinase: 76 kDa.

Background: The c-Abl proto-oncogene encodes a nonreceptor type protein tyrosine kinase that is ubiquitously expressed and highly conserved in metazoan evolution. c-Abl protein is distributed in both the nucleus and the cytoplasm of cells. It has been implicated in regulation of cell proliferation, differentiation, apoptosis, cell adhesion and stress response (1–3). c-Abl kinase activity is increased *in vivo* by diverse physiological stimuli including integrin activation, PDGF stimulation and binding to c-Jun, Nck and RFX1 (2,4). The *in vivo* mechanism of regulation of c-Abl kinase activity is not completely understood. Tyr245 is located in the linker region between the SH2 and catalytic domains, conserved among the Abl family. Phosphorylation of Tyr245 is involved in activation of c-Abl kinase activity (5). Tyr412 is located in the kinase activation loop of c-Abl and phosphorylation of this residue is required for kinase activity (6). Thr735 is in a conserved 14-3-3 protein binding motif, which can be phosphorylated upon stress stimulation or TPA treatment (Wu, J. et al. unpublished data). Phosphorylation of Thr735 in c-Abl may play an important role in regulation of c-Abl localization as well as its function.

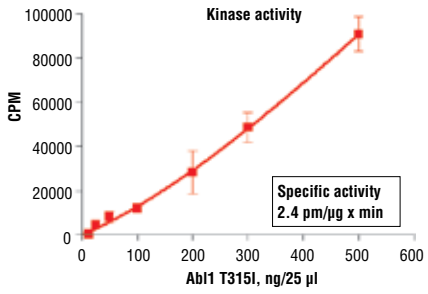

Figure 1. Abl1 T315I kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 3 µM Na-orthovanadate, 1.2 mM DTT, 100 µM ATP, 100 µM Signal Transduction Protein (Tyr160) Biotinylated Peptide #1366 and variable amount of recombinant Abl1 T315I. Reaction mixture incubated at room temperature for 10 minutes.

Imatinib (Gleevec) has been successfully used to treat Bcr-Abl kinase associated chronic myeloid leukemia (CML). The most frequently identified mutation associated with resistance to Gleevec in CML patients is T315I in the Abl kinase domain (7,8).

Source/Purification: The GST-kinase fusion protein was produced using a baculovirus expression system with a construct expressing human Abl1 T315I (Pro118-Ser553) (GenBank Accession No. NM_005157) 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 by screening Tyrosine Kinase Substrate Screening Kit #7450. Phospho-Tyrosine mAb (P-Tyr-100) #9411 was used for detection. The quality of the biotinylated peptides was evaluated by reverse-phase HPLC and by mass spectrometry.

Purified Abl1 T315I kinase was quality controlled for purity by SDS-PAGE followed by Coomassie stain. Abl1 T315I kinase activity 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 Abl1 T315I activity using the Abl1 T315I substrate peptide provided in this kit. Abl1 T315I sensitivity to the inhibitor staurosporine and Gleevec was measured using the Abl1 T315I substrate peptide provided in this kit [Fig.5].

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:

Tyrosine Kinase Substrate Screening Kit #7450

Abl1 T315I Kinase #7906

Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411

Signal Transduction Protein (Tyr160) Biotinylated Peptide #1366

Staurosporine #9953

Background References:

(1) Wang, J.Y. et al. (2000) *Oncogene* 19, 5643–5650.

(2) Van Etten, R.A. et al. (1999) *Trends Cell. Biol.* 9, 179–182.

(3) Danial, N.N. et al. (2000) *Oncogene* 19, 2523–2531.

(4) Shaul, Y. et al. (2000) *Cell Death Differ.* 7, 10–16.

(5) Brasher, B.B. et al. (2000) *J. Biol. Chem.* 275, 35631–35637.

(6) Pluk, H. et al. (2002) *Cell* 108, 247–259.

(7) Fabian, M.A. et al. (2005) *Nat. Biotechnol.* 23, 329–336.

(8) Carter, T.A. et al. (2005) *Proc. Natl. Acad. Sci. USA* 102, 11011-11016.

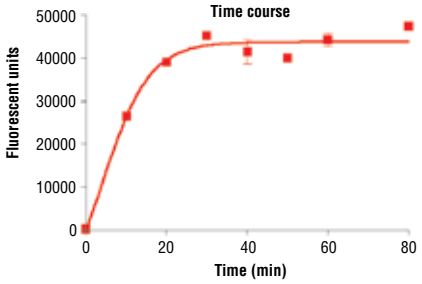

Figure 2. Time course of Abl1 T315I kinase activity: DELFIA® data generated using Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411 to detect phosphorylation of Abl1 T315I substrate peptide (#1366) by Abl1 T315I kinase. In a 50 µl reaction, 50 ng Abl1 T315I and 1.5 µM substrate peptide were used per reaction. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

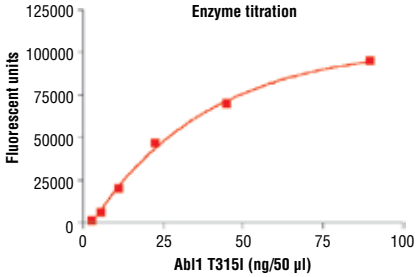

Figure 3. Dose dependence curve of Abl1 T315I kinase activity: DELFIA® data generated using Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411 to detect phosphorylation of substrate peptide (#1366) by Abl1 T315I kinase. In a 50 µl reaction, increasing amounts of Abl1 T315I and 1.5 µM substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

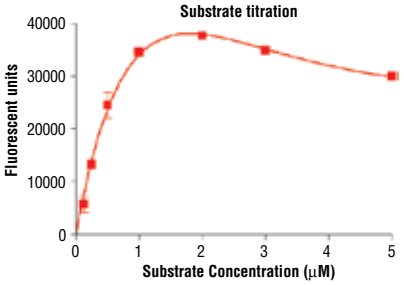

Figure 4. Peptide concentration dependence of Abl1 T315I kinase activity: DELFIA® data generated using Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411 to detect phosphorylation of substrate peptide (#1366) by Abl1 T315I kinase. In a 50 µl reaction, 50 ng of Abl1 T315I and increasing concentrations of substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

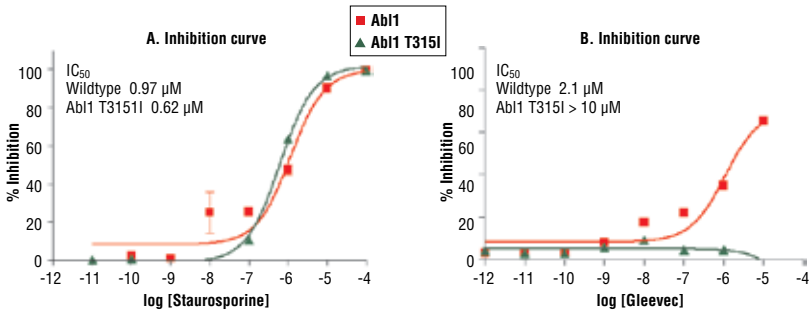

Figure 5. Sensitivities of wild type and mutant Abl1 T315I kinase to Staurosporine (A) and Gleevec (B) were compared: DELFIA® data generated using Phospho-Tyrosine mAb (P-Tyr-100) #9411 to detect phosphorylation of Abl1 substrate peptide (#1366) by the Abl1 kinases. In a 50 µl reaction, 50 ng of kinase, 1.5 µM substrate peptide, 5 µM ATP and increasing concentration of indicated inhibitors were used per reaction well at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

Protocol for HTScan® Abl1 T3151 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 10 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]=40 µM, [substrate]=3 µm).
2. Immediately 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 10 µl of DTT (1.25 M) to 2.5 ml of 4X HTScan® Tyrosine Kinase Buffer (240 mM HEPES pH 7.5, 20 mM $MgCl_2$, 20 mM $MnCl_2$, 12 µM Na_3VO_4) to make DTT/Kinase buffer.
5. Transfer 0.6 ml of DTT/Kinase buffer to each enzyme tube to make 4X reaction cocktail ([enzyme] = 8 ng/µL in 4X reaction cocktail).
6. Incubate 12.5 µl of the 4X reaction cocktail with 12.5 µl/well of prediluted compound of interest (usually around 10 µM) 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

60 mM HEPES pH 7.5
5 mM $MgCl_2$
5 mM $MnCl_2$
3 µM Na_3VO_4
1.25 mM DTT
20 µM ATP
1.5 µM peptide
50 ng Abl1 T3151 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 and 75 µl dH_2O/well to a 96-well streptavidin-coated plate and incubate at room temperature for 60 minutes.
11. *Wash three times with 200 µl/well PBS/T
12. Dilute primary antibody, Phospho-Tyrosine mAb (P-Tyr-100), 1:1000 in PBS/T with 1% BSA. Add 100 µl/well primary antibody.
13. Incubate at room temperature for 60 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