Store at -80°C

#7758

HTScan® Lck Kinase Assay Kit

☑ 100 assays
(96 Well Format)

Cell Signaling TECHNOLOGY®

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Products Included	Products #	Kit Quantity
Phospho-Tyrosine Mouse mAb (P-Tyr-100)	9411	30 µl
HTScan® Tyrosine Kinase Buffer (4X)	9805	15 ml
DTT (1000x, 1.25 M)		80 µl
ATP (10 mM)	9804	1 ml
FHIT (Tyr114) Biotinylated Peptide	1365	1.25 ml
Lck Kinase (recombinant, human)	7487	5 µg

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

Peptide Core Sequence: SIY*EE

Molecular Weights: Peptide substrate, Biotin-FHIT (Tyr114): 2,150 Daltons, GST-Lck Kinase: 84 Daltons.

Background: The Src family of protein tyrosine kinases (including Src, Lyn, Fyn, Yes, Lck, Blk, Hck, etc.) is important in the regulation of growth and differentiation of eukaryotic cells (1). Src activity is regulated by tyrosine phosphorylation at two sites with opposing effects. Phosphorylation of Tyr416 in the activation loop of the kinase domain upregulates enzyme activity. Phosphorylation of Tyr527 in the carboxy-terminal tail by Csk renders the enzyme less active (2).

Lck is essential for T-lymphocyte activation and differentiation (3,4). Phosphorylation of Tyr505 in the carboxy-terminal tail of Lck downregulates its catalytic activity, while phosphorylation of Tyr394 leads to an increase in Lck activity (5).

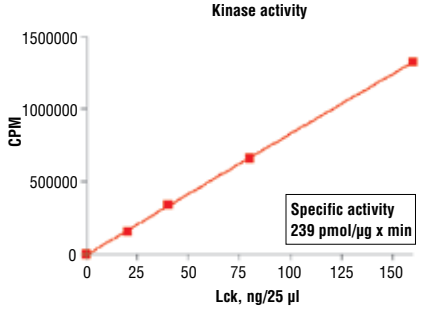

Figure 1. Lck kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 5 mM MgCl2, 0.05 mM DTT, 50 µM ATP, Substrate: Poly (EY 4:1) 400 ng/µL, and variable amount of Lck kinase.

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing a fragment of human Lck (Met1-Pro509) (GenBank accession No. NM_005356) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

Quality Control: FHIT (Tyr114) peptide was selected by using Tyrosine Kinase Substrate Screening Kit #7450 to screen for Lck kinase substrates. Phospho-Tyrosine mAb #9411 was used for detection. The quality of the biotinylated peptides was evaluated by reverse-phase HPLC and by mass spectrometry.

Purified Lck kinase was quality controlled for purity by SDS-PAGE followed by commassie stain. The specific activity of the Lck 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 Lck activity using the Lck substrate peptide provided in this kit. Lck sensitivity to the inhibitor staurosporine was measured using the Lck substrate peptide provided in this kit [Fig.5].

Background References:

(1) Thomas, S.M. and Brugge, J.S. (1997) *Annu. Rev. Cell Dev. Biol.* 13, 513–609.

(2) Hunter, T. (1987) *Cell* 49, 1–4.

(3) Molina, T.J. et al. (1992) *Nature* 357, 161–164.

(4) Straus, D.B. and Weiss, A. (1992) *Cell* 70, 585–593.

(5) Chow, L.M. et al. (1993) *Nature* 365, 156–160.

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.

Enzyme is supplied in 50 mM Tris-HCl, pH 7.5; 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione.

Store at –80°C.

Keep enzymes on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:
Lck Kinase #7487

Tyrosine Kinase Substrate Screening Kit #7450

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

FHIT (Tyr114) Biotinylated Peptide #1365

Phospho-FHIT (Tyr114) Biotinylated Peptide #1462

Staurosporine #9953

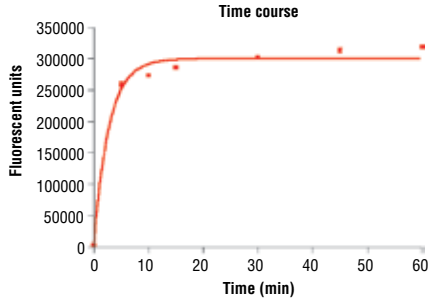

Figure 2. Time course of Lck kinase activity: DELFIA® data generated using Phospho-Tyrosine mAb (P-Tyr-100) #9411 to detect phosphorylation of Lck substrate peptide (#1365) by Lck kinase. In a 50 µl reaction, 50 ng and 1.5 µM substrate peptide were used per reaction. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

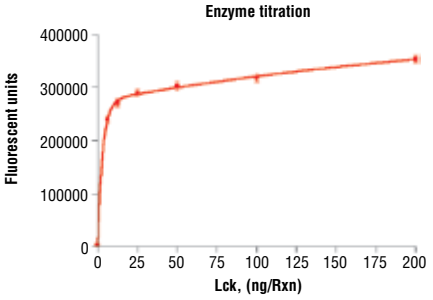

Figure 3. Dose dependence curve of Lck kinase activity: DELFIA® data generated using Phospho-Tyrosine mAb (P-Tyr-100) #9411 to detect phosphorylation of substrate peptide #1365 by Lck kinase. In a 50 µl reaction, increasing amounts of Lck and 1.5 µM substrate peptide were used per reaction well at 25°C for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

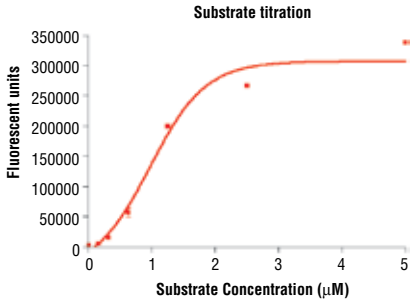

Figure 4. Peptide concentration dependence of Lck kinase activity: DELFIA® data generated using Phospho-Tyrosine mAb (P-Tyr-100) #9411 to detect phosphorylation of substrate peptide (#1365) by Lck kinase. In a 50 µl reaction, 50 ng of Lck and increasing concentrations of substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

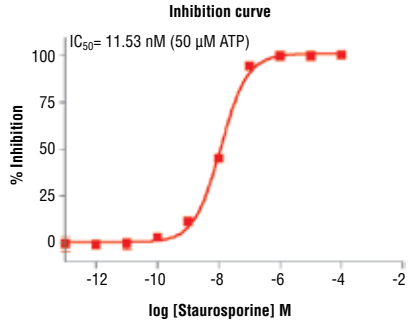

Figure 5. Staurosporine inhibition of Lck kinase activity: DELFIA® data generated using Phospho-Tyrosine mAb (P-Tyr-100) #9411 to detect phosphorylation of Lck substrate peptide (#1365) by Lck kinase. In a 50 µl reaction, 50 ng Lck, 1.5 µM substrate peptide, 50 µM ATP and increasing amounts of staurosporine were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

Protocol for HTScan® Lck 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 1.2 ml of DTT/Kinase buffer to each enzyme tube to make 4X reaction cocktail ([enzyme] = 4 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 Lck 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