TECHNICAL NOTE

U-TRF #16

LANCE *Ultra* Lck Kinase Assay

PerkinElmer, Inc.
Montreal, QC
Canada H3J 1R4

LANCE® *Ultra* TR-FRET Technology

This LANCE *Ultra* kinase assay measures the phosphorylation of a CDK1 peptide substrate at Tyr15.

U*Light*™-CDK1 (Tyr15) Peptide:

- TRF0122-D: 0.5 nmole, 1,000* assay points
- TRF0122-M: 5 nmoles, 10,000* assay points

**0.5 pmol/assay point*

Europium-anti-phospho-tyrosine (PT66) Antibody:

- AD0068: 50 µg, 7,500* assay points
- AD0069: 1 mg, 150,000* assay points

**40 fmol/assay point*

Recognized Motif:

pTyr

Mouse monoclonal antibody directed against phospho-tyrosine.

Peptide Sequence:

CAGAGKIGEGT**Y**GVVYK

Synthetic peptide derived from amino acids 9-20 of human cyclin-dependent kinase 1 (CDK1); phosphorylation site: Tyr15.

LANCE *Ultra* Kinase Assays:

LANCE *Ultra* time-resolved fluorescence resonance energy transfer (TR-FRET) assays use a proprietary europium chelate donor dye, W1024 (Eu), together with U*Light*™, a small molecular weight acceptor dye with a red-shifted fluorescent emission.

In this technical note, we present the optimization of an Lck kinase assay using a U*Light*-labeled peptide substrate. The binding of the Eu-labeled anti-phospho tyrosine PT66 antibody to the CDK1 peptide substrate phosphorylated at Tyr15 brings the Eu donor and U*Light* acceptor dye molecules into close proximity. Upon irradiation at 320 or 340 nm, the energy from the Eu donor is transferred to the U*Light* acceptor dye which, in turn, generates light at 665 nm. The intensity of the light emission is proportional to the level of U*Light* substrate phosphorylation.

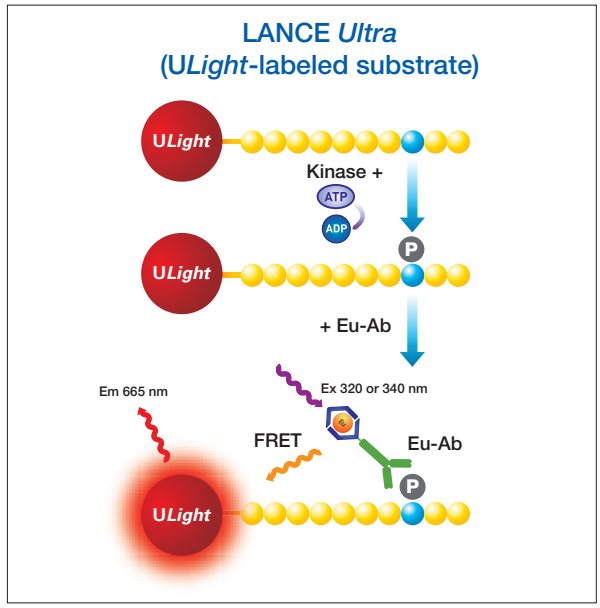

Figure 1. Schematic representation of the LANCE *Ultra* detection of a phosphorylated peptide substrate.

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Development of a Lck Kinase Assay

Reagents needed for this assay:

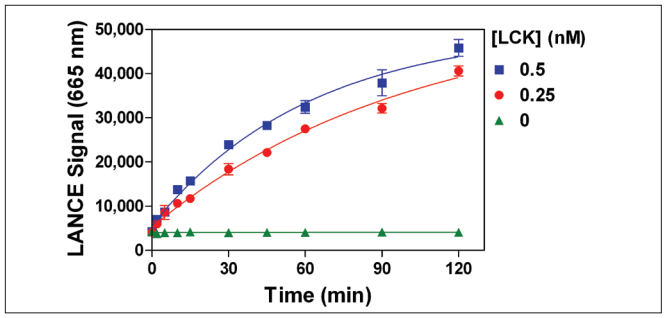
Europium-labeled anti-phospho-tyrosine (PT66) Antibody	PerkinElmer # AD0068/AD0069
U*Light*-CDK1 (Tyr15)	PerkinElmer # TRF0122
Lck active	Enzo Life Sciences # PPK-430
LANCE® Detection Buffer, 10X	PerkinElmer # CR97-100
OptiPlate™-384, white	PerkinElmer # 6007299
TopSeal™-A film	PerkinElmer # 6050195
Kinase Buffer: 50 mM HEPES pH 7.5, 1 mM EGTA, 10 mM $MgCl_2$, 2 mM DTT and 0.01% Tween-20	

Standard Protocol

- Dilute the Lck kinase, ATP, inhibitors and U*Light*-CDK1 (Tyr15) peptide in Kinase Buffer.
- Prepare a 4X Detection Mix by diluting the Eu-anti-phospho-tyrosine antibody to 8 nM in 1X LANCE Detection Buffer.
- Add to the wells of a white OptiPlate-384:
  - 5 µL of Lck enzyme
  - 2.5 µL of inhibitor or Kinase Buffer
  - 2.5 µL of U*Light*-CDK (Tyr15) peptide/ATP mix (for ATP titration, ATP dilutions are added separately in Kinase Buffer).
- Cover the plate with TopSeal-A film and incubate at room temperature (RT).
- Stop kinase reactions by adding 5 µL of 40 mM EDTA prepared in 1X LANCE Detection Buffer (Stop Solution). Leave for 5 min at RT.
- Add 5 µL of 4X Detection Mix (Eu-anti-phospho-tyrosine antibody at a final concentration of 2 nM).
- Cover with TopSeal-A film and incubate for 1 h at RT.
- Remove the TopSeal-A film and read signal with the EnVision® Multilabel Reader in TR-FRET mode (excitation at 320 or 340 nm and emission at 665 nm).

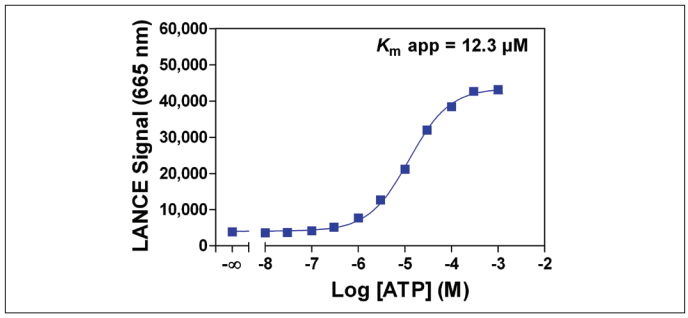
NOTE: *Eu-labeled antibodies and EDTA can be premixed just before use as a 2X concentrated Stop Solution/Detection Mix to minimize the number of liquid handling steps.*

Experiment 1: Enzymatic Titration and Time Course



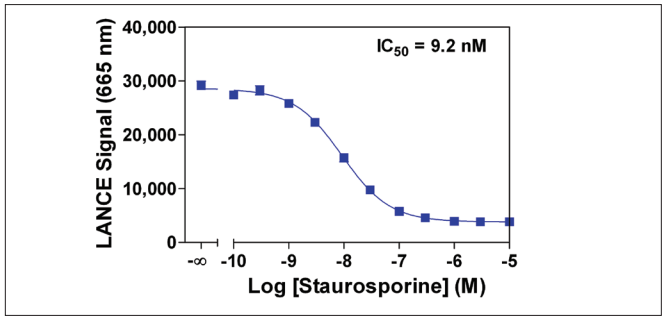
Enzymatic progress curves were produced by incubating Lck enzyme at 0.25 or 0.5 nM with 50 nM U*Light*-CDK1 (Tyr15) peptide and 20 µM ATP. Kinase reactions were terminated at the indicated times by the addition of EDTA. Detection mix was added and signal read after 60 minutes.

Experiment 2: ATP Titration



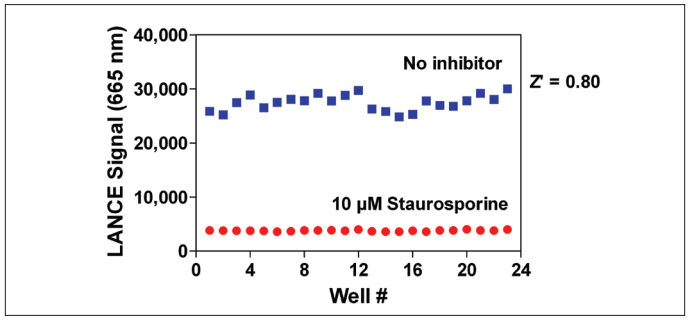
Serial dilutions of ATP ranging from 10 nM to 1 mM were added to 0.5 nM Lck and 50 nM U*Light*-CDK1 (Tyr15) peptide. Kinase reactions were terminated after 60 min by the addition of EDTA.

Experiment 3: Enzyme Inhibition Curve



Serial dilutions of staurosporine ranging from 100 pM to 10 µM (final concentrations in 1% DMSO) were incubated with 0.5 nM Lck, 50 nM U*Light*-CDK1 (Tyr15) peptide and 30 µM ATP. Kinase reactions were terminated after 60 min by the addition of EDTA.

Experiment 4: Z'-factor Determination



Lck enzyme at 0.5 nM was incubated with 50 nM U*Light*-CDK1 (Tyr15) peptide in kinase assay buffer with 30 µM ATP, and with or without 10 µM staurosporine (final concentrations in 1% DMSO). Kinase reactions were terminated after 60 min by the addition of EDTA.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

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