

# Lck Kinase Assay

## ULight-CDK1 (Tyr15) Peptide and Europium-anti-phospho-tyrosine Antibody (PT66)

### Two LANCE Ultra companion products – two convenient sizes!

#### ULight™-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

#### PEPTIDE SEQUENCE:

CAGAGKIGEGTYGVVYK

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

**VALIDATED FOR KINASES:** Lck, Lyn, Src, BTK, EphB4

#### POTENTIAL SUBSTRATE FOR KINASES:

Src subfamily of tyrosine kinases

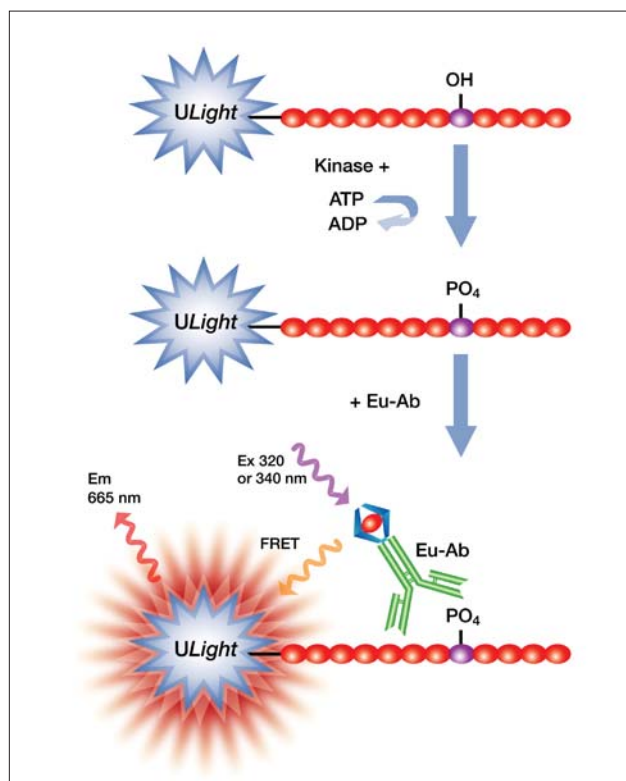
#### Europium-anti-phospho-tyrosine Antibody (PT66):

- 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.



### LANCE Ultra Kinase Assays

LANCE® Ultra time-resolved fluorescence resonance energy transfer (TR-FRET) assays use a proprietary europium chelate donor dye, W-1024 (Eu), with ULight, an innovative small molecular weight acceptor dye with a red-shifted fluorescent emission. In kinase assays, the binding of a Eu-labeled anti-phospho-substrate antibody to the phosphorylated ULight-labeled substrate brings donor and acceptor molecules into close proximity.

After irradiation of the kinase reaction at 320 or 340 nm, the energy from the Eu donor is transferred to the ULight acceptor dye which, in turn, generates light at 665 nm. The intensity of the light emission is proportional to the level of ULight-substrate phosphorylation.

### Development of an Lck Kinase Assay

#### Additional reagents

Lck active	Stressgen #PPK-430
LANCE Detection Buffer, 10X	PerkinElmer # CR97-100
OptiPlate™-384, white	PerkinElmer # 6007299
TopSeal™-A	PerkinElmer # 6005185
Kinase Buffer: 50 mM HEPES pH 7.5, 1 mM EGTA, 10 mM MgCl <sub>2</sub> , 2 mM DTT and 0.01% Tween-20.	

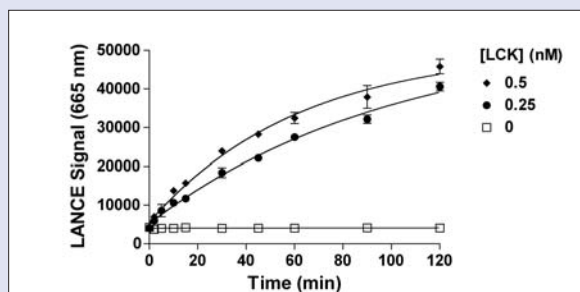
## Suggested Procedure

- Dilute the Lck kinase, ATP, inhibitors and *ULight*-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  $\mu$ L of Lck enzyme
  - 2.5  $\mu$ L of inhibitor or Kinase Buffer
  - 2.5  $\mu$ L of *ULight*-CDK1 (Tyr15) Peptide/ ATP mix (for ATP titration, ATP dilutions are added separately in Kinase Buffer).
- Cover the plate with TopSeal-A and incubate at room temperature (RT).

- Stop kinase reactions by adding 5  $\mu$ L of 40 mM EDTA prepared in 1X Detection Buffer (Stop Solution). Leave for 5 min at RT.
- Add 5  $\mu$ L of 4X Detection Mix (Eu-anti-phospho-tyrosine Antibody at a final concentration of 2 nM).
- Cover with TopSeal-A and incubate for 1 h at RT.
- Remove TopSeal-A and read signal with the EnVision® Multilabel Reader in TR-FRET mode (excitation at 320 nm and emission at 665 nm).

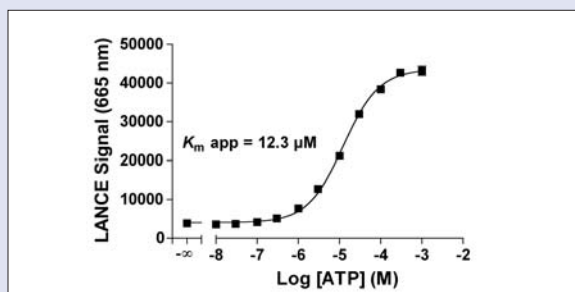
**NOTE:** Eu-labeled antibodies and EDTA can be premixed before use as a 2X concentrated Stop Solution/Detection Mix to minimize the number of liquid handling steps.

### Experiment 1: Enzymatic Time Course



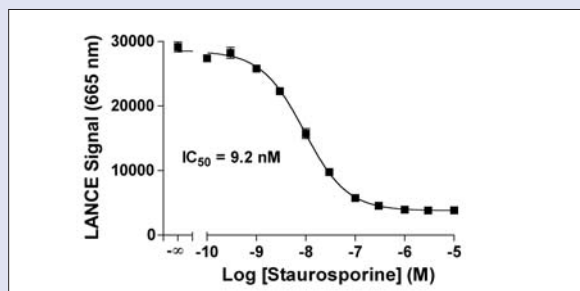
Lck enzyme at 0.25 nM or 0.5 nM was incubated with 50 nM *ULight*-CDK1 (Tyr15) peptide and 20  $\mu$ M ATP. Kinase reactions were terminated after 0 to 120 min by the addition of EDTA.

### Experiment 2: ATP Titration



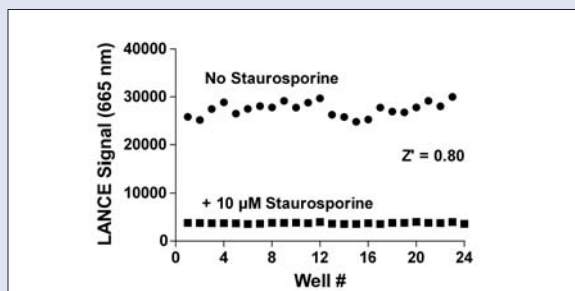
Serial dilutions of ATP ranging from 10 nM to 1 mM were added to 0.5 nM Lck and 50 nM *ULight*-CDK1 (Tyr15) peptide. Kinase reactions were terminated after 60 min by the addition of EDTA.

### Experiment 3: Enzyme Inhibition Curve



Serial dilutions of staurosporine ranging from 100  $\mu$ M to 10  $\mu$ M (final concentrations in 1% DMSO) were incubated with 0.5 nM Lck, 50 nM *ULight*-CDK1 (Tyr15) peptide and 30  $\mu$ M 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 *ULight*-CDK1 (Tyr15) peptide and 30  $\mu$ M ATP with or without 10  $\mu$ M staurosporine (final concentrations in 1% DMSO). Kinase reactions were terminated after 60 min by the addition of EDTA.