TECHNICAL NOTE

LANCE *Ultra* Aurora A Kinase Assay

U-TRF #6

PerkinElmer, Inc.

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LANCE® *Ultra* TR-FRET Technology

This LANCE *Ultra* kinase assay measures the phosphorylation of a PLK peptide substrate at Ser137.

Europium-anti-phospho-PLK (Ser137) Antibody:

- TRF0203-D: 10 µg, 1,562 assay points*
- TRF0203-M: 100 µg, 15,562 assay points*

**40 fmol/assay point*

- RECOGNIZED MOTIF: RRR**<u>pS</u>**LLE
- Europium-labeled rabbit polyclonal antibody recognizing phospho-Ser137 in human polo-like kinase (PLK)

U*Light*™-PLK (Ser137) Peptide:

- TRF0110-D: 0.5 nmole, 1,000 assay points*
- TRF0110-M: 5 nmoles, 10,000 assay points*

**0.5 pmol/assay point*

- CORE SEQUENCE MOTIF: RRR**<u>S</u>**LLE
  - Synthetic peptide containing the residues surrounding Ser137 of polo-like kinase (PLK)
  - Phosphorylation site: Ser137

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 a Aurora kinase assay using a U*Light*-labeled peptide substrate. The binding of an Eu-labeled antibody directed against Ser137 phosphorylation of the PLK peptide substrate 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.

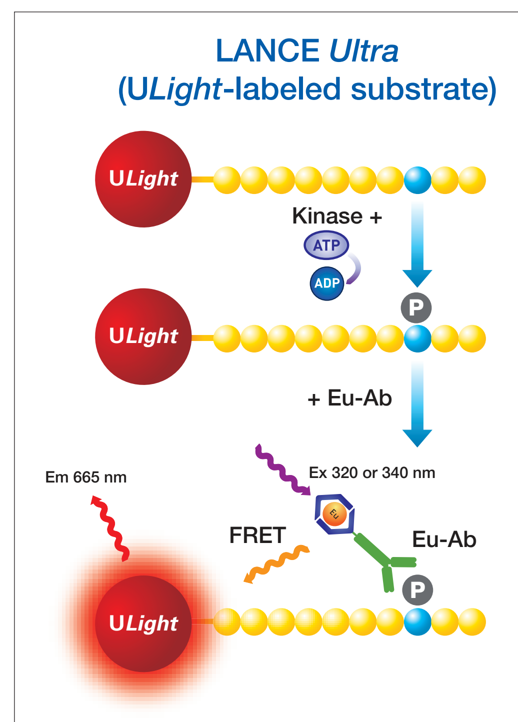

Figure 1. Schematic representation of the LANCE *Ultra* detection of a phosphorylated peptide substrate.

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Development of an Aurora A Kinase Assay:

Reagents needed for this assay:

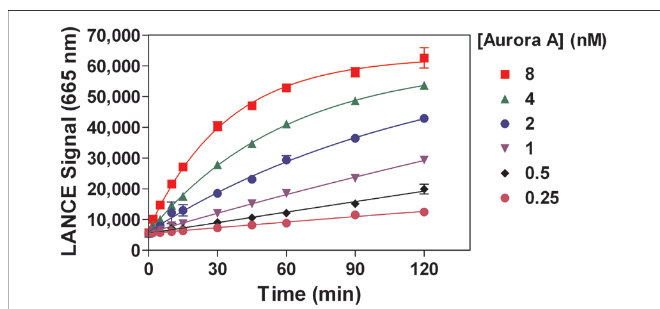
Europium-labeled anti-phospho-PLK (Ser137) Antibody	PerkinElmer # TRF0203
U*Light*-PLK (Ser137) Peptide	PerkinElmer # TRF0110
Aurora A, active	Millipore # 14-511
LANCE Detection Buffer, 10X	PerkinElmer # CR97-100
OptiPlate™-384, white	PerkinElmer # 6007299
TopSeal-A™ film	PerkinElmer # 6050195
Kinase 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 Aurora A kinase, ATP, inhibitors and U*Light*-PLK peptide in Kinase Buffer.
- Prepare a 4X Detection Mix by diluting the Eu-anti-phospho-PLK antibody to 8 nM in 1X LANCE Detection Buffer.
- Add to the wells of a white OptiPlate-384:
  - 5 µL of Aurora A enzyme
  - 2.5 µL of inhibitor or Kinase Buffer
  - 2.5 µL of U*Light*-PLK 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. Leave for 5 min at RT.
- Add 5 µL of Detection Mix (Eu-anti-phospho-PLK antibody at a final concentration of 2 nM).
- Cover with the TopSeal-A film and incubate for 1 h at RT.
- Remove 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).

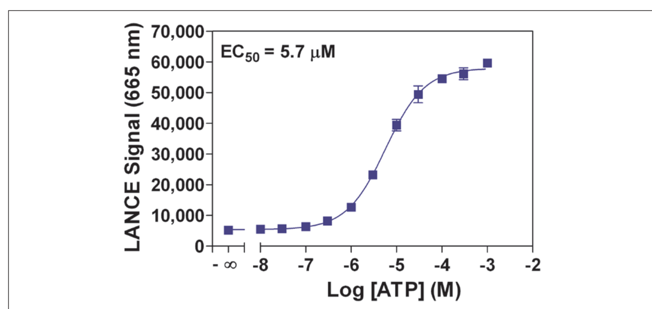
NOTE: Eu-labeled antibodies and EDTA can be premixed just before use as a 2X concentrated Stop/Detection mix to minimize the number of liquid handling steps.

Experiment 1: Enzymatic Titration and Time-Course



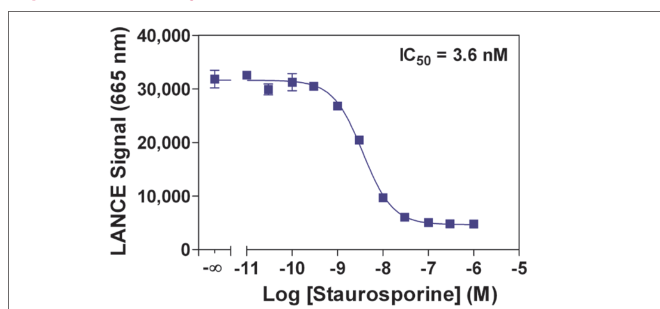
Enzymatic progress curves were produced by incubating Aurora A enzyme at final concentrations ranging from 0.25 to 8 nM with 50 nM U*Light*-PLK peptide and 20 µM ATP. Kinase reactions were terminated at the indicated times by the addition EDTA. Detection mix was added and signal read after 60 minutes.

Experiment 2: ATP Titration



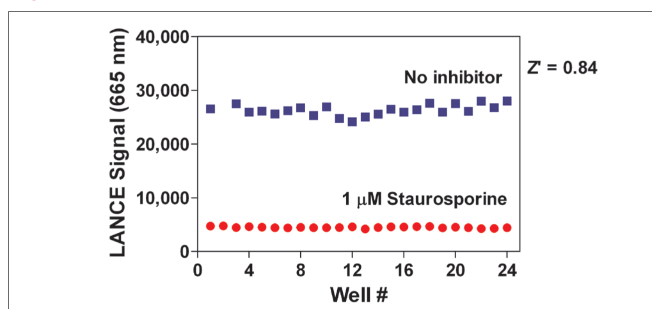
Serial dilutions of ATP ranging from 10 nM to 1 mM were added to 4 nM Aurora A and 50 nM of U*Light*-PLK peptide. Kinase reactions were terminated after 60 min by the addition of EDTA.

Experiment 3: Enzyme Inhibition



Serial dilutions of staurosporine ranging from 10 pM to 1 µM (final concentrations in 2% DMSO) were incubated with 4 nM Aurora A, 50 nM U*Light*-PLK peptide and 5 µM ATP. Kinase reactions were terminated after 60 min by the addition of EDTA.

Experiment 4: Z′-factor Determination



Aurora A enzyme at 4 nM was incubated with 50 nM U*Light*-PLK peptide and 5 µM ATP with or without 1 µM staurosporine (final concentrations in 2% DMSO). Kinase reactions were terminated after 60 min by the addition of EDTA.

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