TECHNICAL NOTE

U-TRF #22

LANCE *Ultra* Aurora B Kinase Assay

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LANCE® *Ultra* TR-FRET Technology

This LANCE *Ultra* kinase assay measures the phosphorylation of a Histone H3 peptide substrate at Ser10.

U*Light*™-Histone H3 (Thr3/Ser10) Peptide:

- TRF0125-D: 0.5 nmole, 1,000* assay points
- TRF0125-M: 5 nmoles, 10,000* assay points

**0.5 pmol/assay point*

Europium-anti-phospho-Histone H3 (Ser10) Antibody:

- TRF0210-D: 10 µg, 1,562* assay points
- TRF0210-M: 100 µg, 15,625* assay points

**40 fmol/assay point*

Recognized Motif:

TKQTARKp<u>S</u>TGGKAPR

Europium-labeled mouse monoclonal antibody recognizing phospho-Ser10 in human Histone H3.

Peptide Sequence:

AR<u>T</u>KQTARK<u>S</u>TGGKAPRKQLAGCG

Synthetic peptide containing the residues surrounding Thr3 and Ser10 of human Histone H3; phosphorylation sites: Thr3 and Ser10.

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 B kinase assay using a U*Light*-labeled peptide substrate. The binding of a Eu-labeled antibody directed against Ser10 phosphorylation of the Histone H3 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.

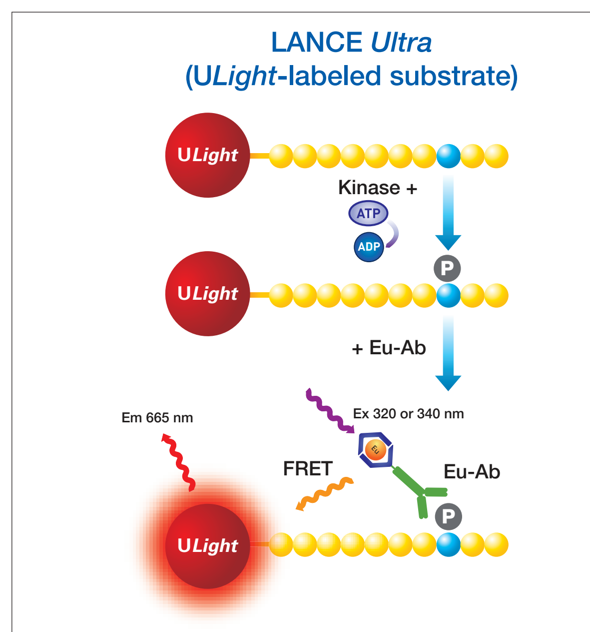

Figure 1. Schematic representation of the LANCE *Ultra* detection of a phosphorylated peptide substrate.

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For the Better

Development of Aurora B Kinase Assay

Reagents needed for this assay:

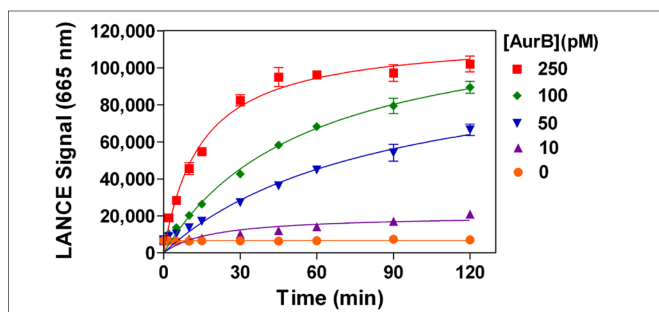
Reagent	Source
Europium-labeled anti-phospho-Histone H3 (Ser10) Antibody	PerkinElmer # TRF0210
U*Light*™-Histone H3 (Thr3/Ser10)	PerkinElmer # TRF0125
Aurora B active	Carna # 05-102
LANCE® Detection Buffer, 10X	PerkinElmer # CR97-100
OptiPlate™-384, white	PerkinElmer # 6007299
TopSeal™-A film	PerkinElmer # 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 B kinase, ATP, inhibitors and U*Light*-Histone H3 (Thr3/Ser10) Peptide in Kinase Buffer.
- Prepare a 4X Detection Mix by diluting the Eu-anti-phospho-Histone H3 (Ser10) antibody to 8 nM in 1X LANCE Detection Buffer.
- Add to the wells of a white OptiPlate-384:
  - 5 µL of Aurora B enzyme
  - 2.5 µL of inhibitor or Kinase Buffer
  - 2.5 µL of U*Light*-Histone H3 (Thr3/Ser10) Peptide/ATP mix (for ATP titration, ATP dilutions are added separately in Kinase Buffer).
- Cover the plate with TopSeal-A film and incubate for 60 min 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 Detection Mix (Eu-anti-phospho-Histone H3 (Ser10) 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).

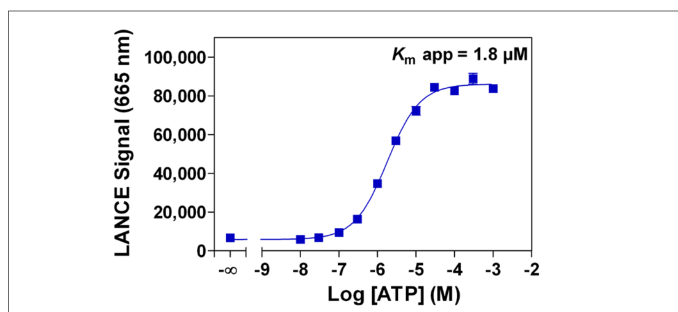
NOTE: *Eu-labeled antibodies and EDTA can be premixed just before use as a 2X concentrated Stop Solution/Detection Mix to minimize the number of liquid handling steps.*

Experiment 1: Enzymatic Titration and Time Course



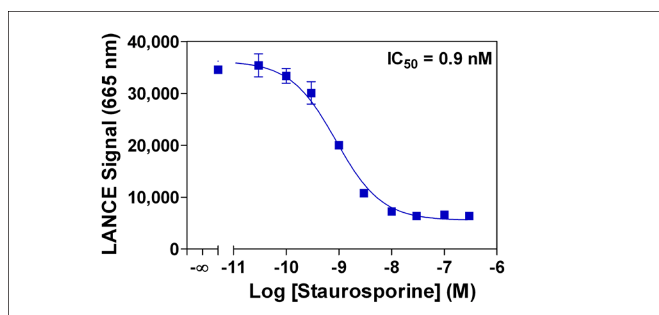
Enzymatic progress curves were produced by incubating Aurora B enzyme at final concentrations ranging from 10 to 250 pM with 50 nM U*Light*-Histone H3 (Thr3/Ser10) Peptide and 20 µM ATP. Kinase reactions were terminated at the indicated times by the addition of EDTA. Detection mix was added and signal read after 60 minutes.

Experiment 2: ATP Titration



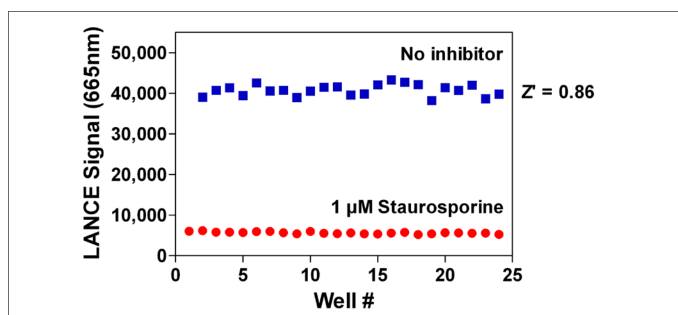
Serial dilutions of ATP ranging from 10 nM to 1 mM were added to 100 pM Aurora B and 50 nM U*Light*-Histone H3 (Thr3/Ser10) peptide. Kinase reactions were terminated after 60 min by the addition of EDTA.

Experiment 3: Enzyme Inhibition Curve



Serial dilutions of staurosporine ranging from 30 pM to 300 nM (final concentrations in 2% DMSO) were incubated with 100 pM Aurora B, 50 nM U*Light*-Histone H3 (Thr3/Ser10) Peptide and 2 µM ATP. Kinase reactions were terminated after 60 min by the addition of EDTA.

Experiment 4: Z′-factor Determination



Aurora B enzyme at 100 pM was incubated with 50 nM U*Light*-Histone H3 (Thr3/Ser10) Peptide in kinase assay buffer with 2 µM ATP, and 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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