TECHNICAL NOTE

U-TRF #21

LANCE *Ultra* Haspin Kinase Assay

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LANCE® *Ultra* TR-FRET Technology

This LANCE *Ultra* kinase assay measures the phosphorylation of a Histone 3 peptide substrate at Thr3.

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 3 (Thr3) Antibody:

- TRF0211-D: 10 µg, 1,562* assay points
- TRF0211-M: 100 µg, 15,625* assay points

**40 fmol/assay point*

Recognized Motif:

Europium-labeled rabbit monoclonal antibody recognizing phospho-Thr3 in human Histone H3.

Peptide Sequence:

ARTKQTARKSTGGKAPRKQLAGCG

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 Haspin kinase assay using a U*Light*-labeled peptide substrate. The binding of a Eu-labeled anti-phospho-Histone H3 (Thr3) antibody to the Histone H3 peptide substrate at Thr3 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.

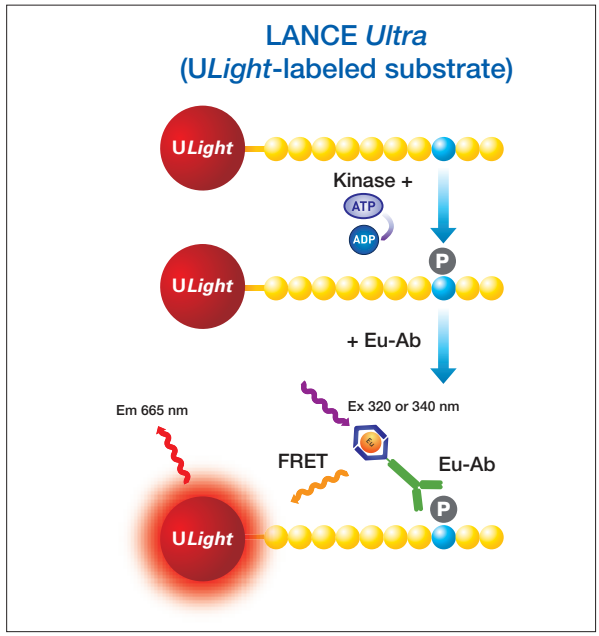

Figure 1. Schematic representation of the LANCE *Ultra* detection of a phosphorylated peptide substrate.

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Development of Haspin Kinase Assay

Reagents needed for this assay:

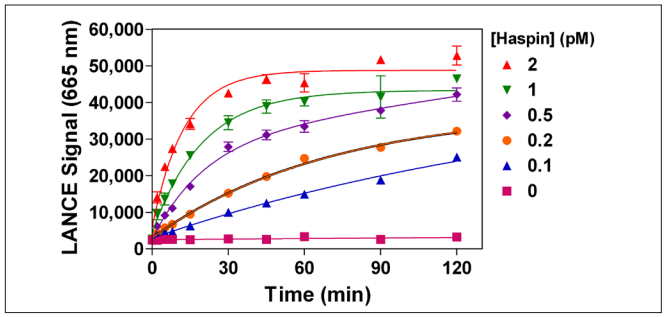
Europium-anti-phospho-Histone H3 (Thr3) Antibody	PerkinElmer # TRF0211
U*Light*™-Histone H3 (Thr3/Ser10) Peptide	PerkinElmer # TRF0125
Haspin active	Carna # 05-111
LANCE® Detection Buffer, 10X	PerkinElmer # CR97-100
OptiPlate™-384, white	PerkinElmer # 6007299
TopSeal™-A film	PerkinElmer # 6050195
Kinase 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 Haspin 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 (Thr3) antibody to 8 nM in 1X LANCE Detection Buffer.
- Add to the wells of a white OptiPlate-384:
  - 5 µL of Haspin 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 at room temperature (RT).
- Stop kinase reactions by adding 5 µL of 24 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 (Thr3) 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).

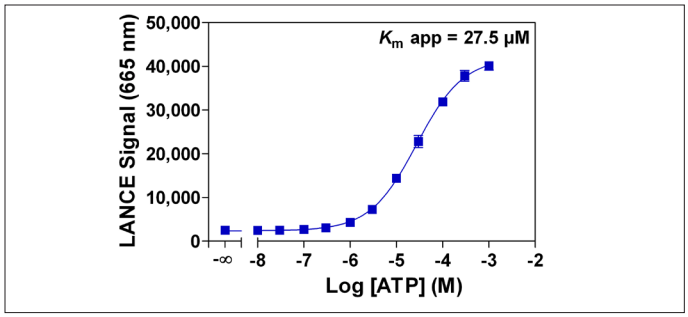
NOTE: *Eu-labeled antibodies and EDTA can be premixed just before use as a 2X concentrated Stop Solution/Detection Mix to minimize the number of liquid handling steps.*

Experiment 1: Enzymatic Titration and Time Course



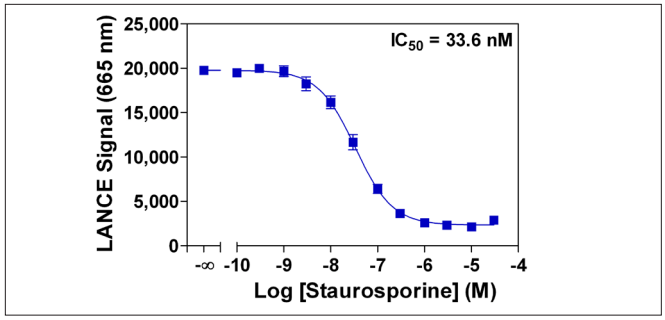
Enzymatic progress curves were produced by incubating Haspin enzyme at concentrations ranging from 0.1 to 2 pM with 50 nM U*Light*-Histone H3 (Thr3/Ser10) peptide and 100 µM ATP. Kinase reactions were terminated at the indicated times by the addition of EDTA. Detection mix was added and signal read after 60 minutes.

Experiment 2: ATP Titration



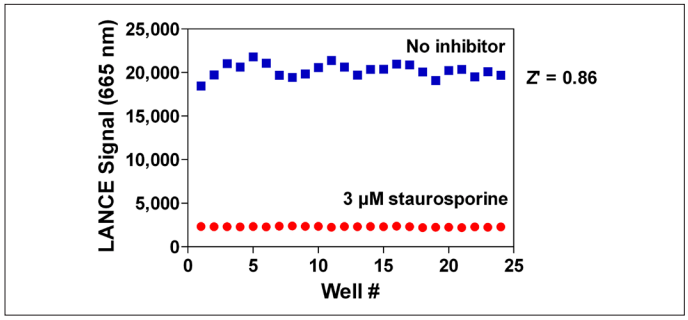
Serial dilutions of ATP ranging from 10 nM to 1 mM were added to 0.5 pM Haspin enzyme and 50 nM U*Light*-Histone H3 (Thr3/Ser10) peptide. Kinase reactions were terminated after 60 min by the addition of EDTA.

Experiment 3: Enzyme Inhibition Curve



Serial dilutions of staurosporine ranging from 0.1 nM to 30 µM (final concentrations in 1% DMSO) were incubated with 0.5 pM Haspin enzyme, 50 nM U*Light*-Histone H3 (Thr3/Ser10) peptide and 30 µM ATP. Kinase reactions were terminated after 60 min by the addition of EDTA.

Experiment 4: Z'-factor Determination



Haspin enzyme at 0.5 pM was incubated with 50 nM U*Light*-Histone H3 (Thr3/Ser10) peptide in kinase assay buffer with 30 µM ATP, and with or without 3 µM staurosporine (final concentrations in 1% DMSO). Kinase reactions were terminated after 60 min by the addition of EDTA.

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