TECH NOTE *U*-TRF #22

Aurora B Kinase Assay

U*Light*-Histone H3 (Thr3/Ser10) Peptide & Europium-anti-phospho-Histone H3 (Ser10) Antibody

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

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*

PEPTIDE SEQUENCE:
ARTKQTARKSTGGKAPRKQLAGCG

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

VALIDATED FOR KINASE: Aurora B, CRIK, MRCKα, WNK2, WNK3.

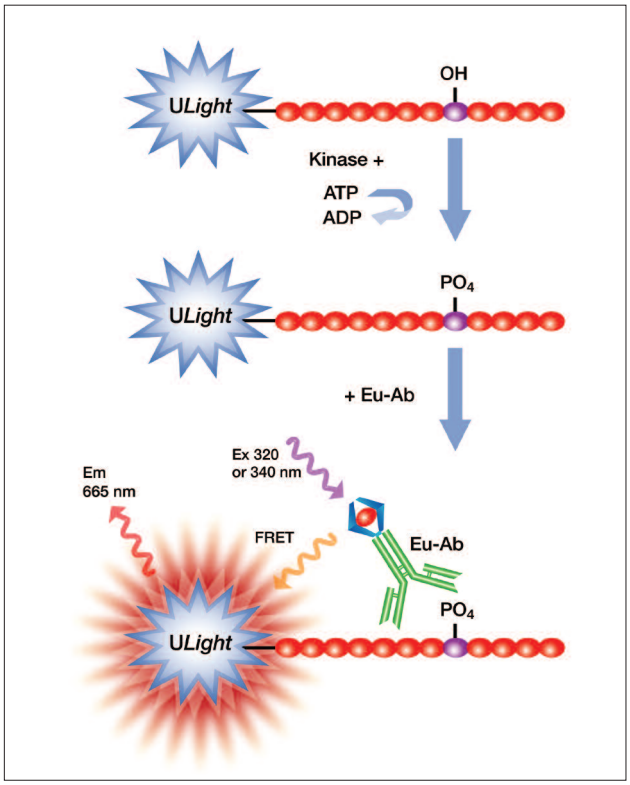
Europium-anti-phospho-Histone H3 (Ser10) Antibody:

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

**40 fmol/assay point*

RECOGNIZED MOTIF:
TKQTARKpSTGGKAPR

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



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 U*Light*, 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 U*Light*-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 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.

Development of Aurora B Kinase Assay

Additional reagents

Aurora B active	Carna # 05-102
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 $MgCl_2$, 2 mM DTT and 0.01% Tween-20

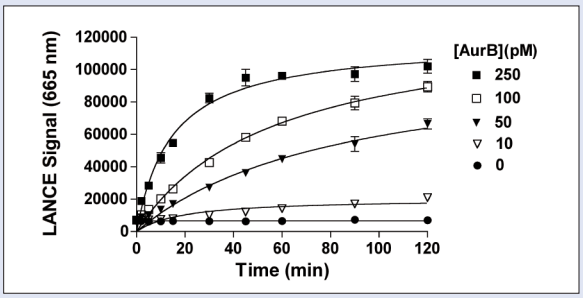
PerkinElmer® precisely.

Suggested procedure

- 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 a TopSeal-A and incubate for 60 min at room temperature (RT).
- Stop kinase reactions by adding 5 µL of 40 mM EDTA prepared in 1X 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 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 & emission at 665 nm).

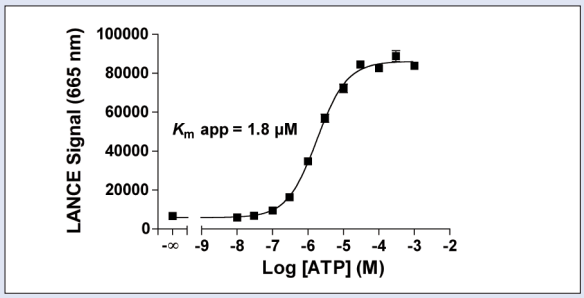
NOTE: Eu-labeled antibodies and EDTA can be premixed before use as a 2X concentrated Stop Solution/Detection mix to minimize the number of liquid handling steps.

Experiment 1: Enzymatic Time Course



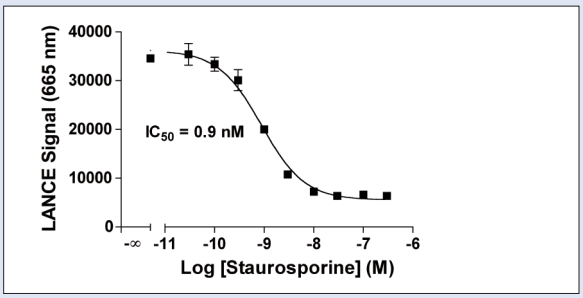
Aurora B enzyme was incubated at concentrations ranging from 10 to 250 pM with 50 nM U*Light*-Histone H3 (Thr3/Ser10) Peptide and 20 µM ATP. Kinase reactions were terminated after 0 to 120 min by the addition of EDTA.

Experiment 2: ATP Titration



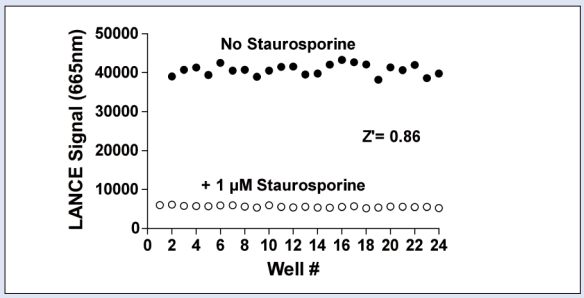
Serial dilutions of ATP ranging from 10 nM to 1 mM were added to 100 pM Aurora B and 50 nM U*Light*-Histone H3 (Thr3/Ser10) Peptide. Kinase reactions were terminated after 60 min by the addition of EDTA.

Experiment 3: Enzyme Inhibition Curve



Serial dilutions of staurosporine ranging from 0.03 nM to 0.3 µM (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 and 2 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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