TECHNICAL NOTE

U-TRF #23

LANCE *Ultra* JNK1α1 Kinase Assay

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LANCE® *Ultra* TR-FRET Technology

This LANCE *Ultra* kinase assay measures the phosphorylation of human ATF-2 protein at Thr53.

U*Light*™-anti-GST:

- TRF0104-D: 1 nmole, 1,000 assay points*
- TRF0104-M: 10 nmoles, 10,000 assay points*
- TRF0104-R: 100 nmoles, 100,000 assay points*

** 100 fmol/assay point*

Europium-anti-phospho-ATF-2 (Thr53) Antibody:

- TRF0212-D: 10 µg, 1,562 assay points*
- TRF0212-M: 100 µg, 15,625 assay points*

**40 fmol/assay point*

Recognized Protein:

U*Light*-labeled goat polyclonal antibody recognizing GST fusion proteins.

Recognized Motif:

IVADQTPp**T**PTRFLKN

Europium-labeled rabbit monoclonal antibody recognizing phospho-Thr53 in human ATF-2 (also referred as Thr71 in some publications).

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 kinase assay configuration, the phosphorylated GST-tag fusion substrate is detected by both the U*Light*-labeled anti-GST and Eu-labeled anti-phospho-substrate antibodies, which brings donor and acceptor dyes 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.

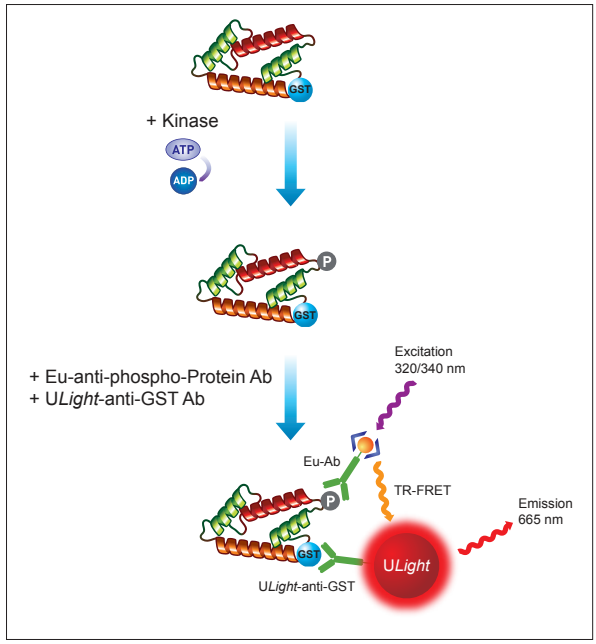

Figure 1. Schematic representation of the LANCE *Ultra* detection of a phosphorylated protein.

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For the Better

Development of a JNK1α1 Kinase Assay

Reagents needed for this assay:

Europium-labeled anti-phospho-ATF-2 (Thr53) Antibody	PerkinElmer # TRF0212
U*Light*-anti-GST	PerkinElmer # TRF0104
JNK1α1/SAP1c, active	Millipore # 14-327
ATF-2 fusion protein (GST-tagged)	CST # 9224
LANCE® Detection Buffer, 10X	PerkinElmer # CR97-100
OptiPlate™-384, white	PerkinElmer # 6007299
TopSeal™-A film	PerkinElmer # 6050195

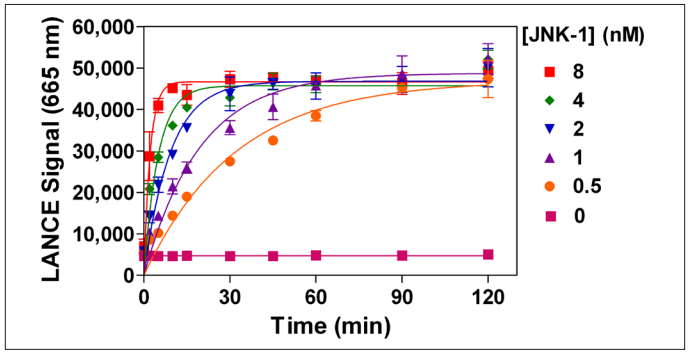
Kinase Buffer: 50 mM HEPES pH 7.5, 1 mM EGTA, 10 mM $MgCl_2$, 2 mM DTT and 0.01% Tween-20.

Standard Protocol

- Dilute the JNK1α1 kinase, ATP, inhibitors and ATF-2 fusion protein in Kinase Buffer.
- Prepare a 4X Detection Mix by diluting the Eu-anti-phospho-ATF-2 Antibody to 8 nM and U*Light*-anti-GST to 20 nM in 1X LANCE Detection Buffer.
- Add to the wells of a white OptiPlate-384:
  - 5 µL of JNK1α1 enzyme
  - 2.5 µL of inhibitor or Kinase Buffer
  - 2.5 µL of ATF-2 fusion protein/ATP mix (for ATP titration, ATP dilutions are added separately in Kinase Buffer).
- Cover the plate with TopSeal-A film and incubate for 30 min at room temperature (RT).
- Stop kinase reaction 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-ATF-2 Antibody at final concentration of 2 nM and U*Light*-anti-GST at final concentration of 5 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 Plate Reader in TR-FRET mode (excitation at 320 or 340 nm and emission at 665 nm).

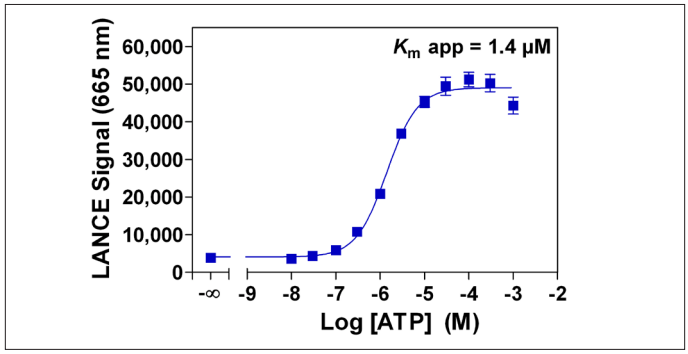
NOTE: *Eu-labeled antibodies and EDTA can be premixed just before use as a 2X concentrated Stop Solution/Detection Mix to minimize the number of liquid handling steps.*

Experiment 1: Enzymatic Titration and Time Course



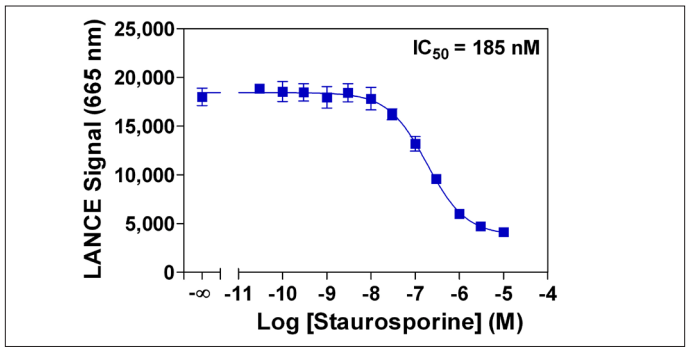
Enzymatic progress curves were produced by incubating JNK1α1 enzyme at concentrations ranging from 0.5 to 8 nM with 30 nM ATF-2 fusion protein and 20 µM ATP. Kinase reactions were terminated at the indicated times by the addition of EDTA. Detection mix was added and signal read after 60 minutes.

Experiment 2: ATP Titration



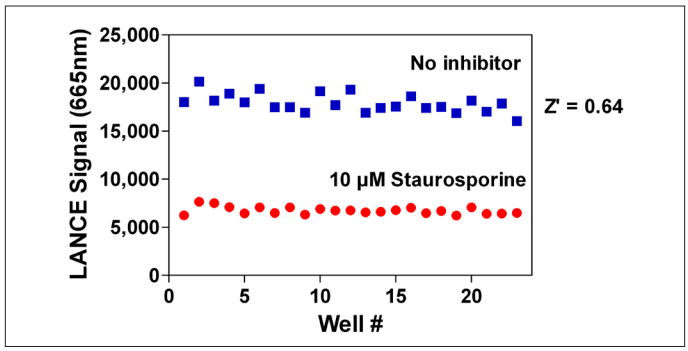
Serial dilutions of ATP ranging from 10 nM to 1 mM were added to 1 nM JNK1α1 and 30 nM ATF-2 fusion protein. Kinase reactions were terminated after 30 min by the addition of EDTA.

Experiment 3: Enzyme Inhibition Curve



Serial dilutions of staurosporine ranging from 0.03 nM to 10 µM (final concentrations in 2% DMSO) were incubated with 1 nM JNK1α1, 30 nM ATF-2 fusion protein and 1 µM ATP. Kinase reactions were terminated after 30 min by the addition of EDTA.

Experiment 4: Z'-factor Determination



Kinase reactions with 1 nM JNK1α1, 30 nM ATF-2 fusion protein and 1 µM ATP were incubated in kinase assay buffer in the presence or absence of 10 µM staurosporine (final concentrations in 2% DMSO). Reactions were terminated after 30 min by the addition of EDTA.

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