

# LANCE *Ultra* IKK $\beta$ Kinase Assay

U-TRF #7

## LANCE<sup>®</sup> *Ultra* TR-FRET Technology

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This LANCE *Ultra* kinase assay measures the phosphorylation of IkappaB-alpha peptide substrate at Ser32/36.

### Europium-anti-phospho-IkappaB-alpha (Ser32/36) Antibody:

- TRF0206-D: 10  $\mu$ g, 1,562 assay points\*
  - TRF0206-M: 100  $\mu$ g, 15,625 assay points\*
- \*40 fmol/assay point

- RECOGNIZED MOTIF: RHDpSGLDpSM
- Europium-labeled mouse monoclonal antibody recognizing phospho-Ser32/36 in human IkappaB-alpha

### ULight<sup>™</sup>-IkappaB-alpha (Ser32/36) Peptide:

- TRF0113-D: 0.5 nmole, 1,000 assay points\*
  - TRF0113-M: 5 nmoles, 10,000 assay points\*
- \*0.5 pmol/assay point
- CORE SEQUENCE MOTIF: RHDSGLDSM
    - Synthetic peptide containing the residues surrounding Ser32/36 of human IkappaB-alpha
    - Phosphorylation site: Ser32/36

### 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 ULight<sup>™</sup>, a small molecular weight acceptor dye with a red-shifted fluorescent emission.

In this technical note, we present the optimization of a IKK $\beta$  kinase assay using a ULight-labeled peptide substrate. The binding of an Eu-labeled antibody directed against Ser32/36 phosphorylation of the IkappaB-alpha peptide substrate brings the Eu donor and ULight acceptor dye molecules into close proximity. Upon irradiation at 320 or 340 nm, the energy from the Eu donor is transferred to the ULight acceptor dye which, in turn, generates light at 665 nm. The intensity of the light emission is proportional to the level of ULight substrate phosphorylation.

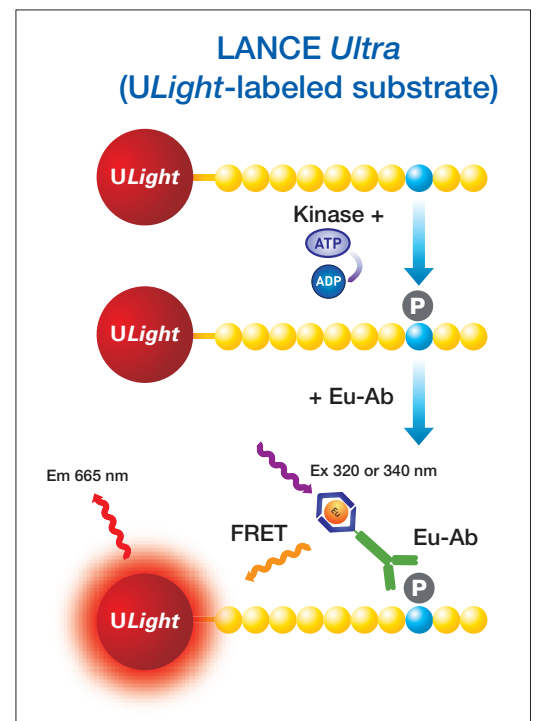


Figure 1. Schematic representation of the LANCE *Ultra* detection of a phosphorylated peptide substrate.

## Development of IKK $\beta$ Kinase Assay:

### Reagents needed for this assay:

Europium-labeled anti-phospho-IkappaB-alpha (Ser32/36) Antibody	PerkinElmer # TRF0206
ULight-IkappaB-alpha (Ser32/36) Peptide	PerkinElmer # TRF0113
IKK $\beta$ , active	Millipore # 14-485
LANCE Detection Buffer, 10X	PerkinElmer # CR97-100
OptiPlate™-384, white	PerkinElmer # 6007299
TopSeal-A™ film	PerkinElmer # 6050195

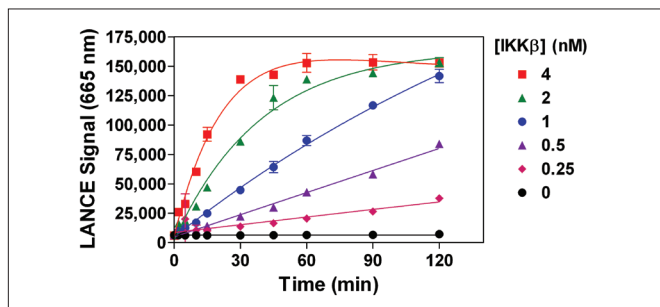
Kinase Buffer: 50 mM HEPES, pH 7.5, 1 mM EGTA, 10 mM MgCl<sub>2</sub>, 2 mM DTT and 0.01% Tween-20

### Standard Protocol

- Dilute IKK $\beta$  kinase, ATP, inhibitors and ULight-IkappaB-alpha peptide in Kinase Buffer.
- Prepare a 4X Detection Mix by diluting the Eu-anti-phospho-IkappaB-alpha antibody to 8 nM in 1X LANCE Detection Buffer.
- Add to the wells of a white OptiPlate-384:
  - 5  $\mu$ L of IKK $\beta$  enzyme
  - 2.5  $\mu$ L of inhibitor or Kinase Buffer
  - 2.5  $\mu$ L of ULight-IkappaB-alpha 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  $\mu$ L of 40 mM EDTA prepared in 1X LANCE Detection Buffer. Leave for 5 min at RT.
- Add 5  $\mu$ L of Detection Mix (Eu-anti-phospho-IkappaB-alpha antibody at a final concentration of 2 nM).
- Cover with the 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).

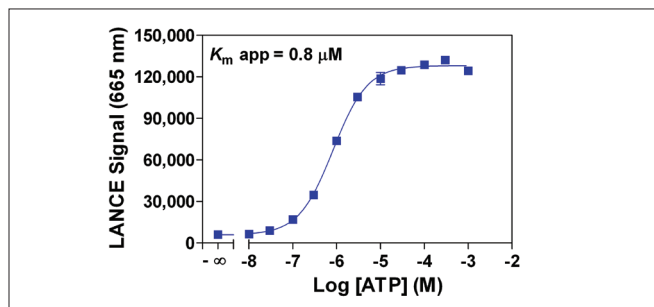
*NOTE: Eu-labeled antibodies and EDTA can be premixed just before use as a 2X concentrated Stop/Detection mix to minimize the number of liquid handling steps.*

### Experiment 1: Enzymatic Titration and Time-Course



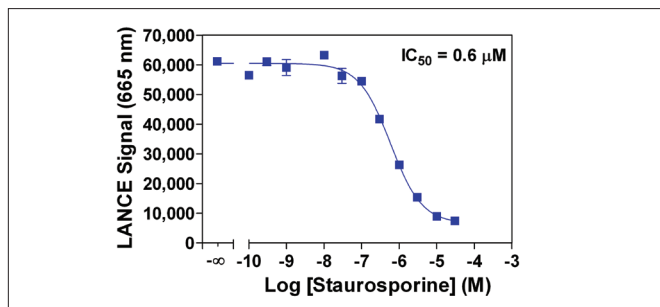
Enzymatic progress curves were produced by incubating IKK $\beta$  enzyme at concentrations ranging from 0.25 to 4 nM with 50 nM ULight-IkappaB-alpha peptide and 20  $\mu$ M ATP. Kinase reactions were terminated at the indicated times by the addition of EDTA. Detection mix was added and signal read after 60 minutes.

### Experiment 2: ATP Titration



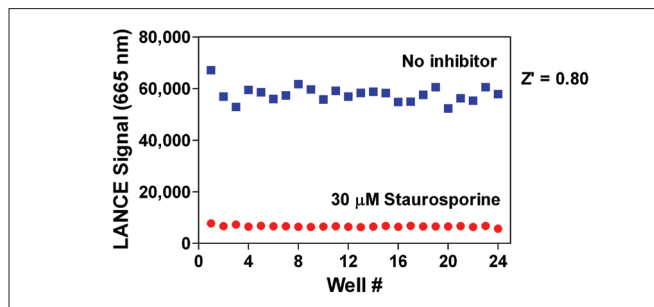
Serial dilutions of ATP ranging from 10 nM to 1 mM were added to 2 nM IKK $\beta$  and 50 nM of ULight-IkappaB-alpha peptide. Kinase reactions were terminated after 60 min by the addition of EDTA.

### Experiment 3: Enzyme Inhibition



Serial dilutions of staurosporine ranging from 0.1 nM to 30  $\mu$ M (final concentrations in 1% DMSO) were incubated with 2 nM IKK $\beta$ , 50 nM ULight-IkappaB-alpha peptide and 1  $\mu$ M ATP. Kinase reactions were terminated after 60 min by the addition of EDTA.

### Experiment 4: Z'-factor Determination



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