TECHNICAL NOTE

LANCE *Ultra* PAK2 Kinase Assay

U-TRF #8

PerkinElmer, Inc.

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LANCE® *Ultra* TR-FRET Technology

This LANCE *Ultra* kinase assay measures the phosphorylation of a Tyrosine Hydroxylase Peptide at Ser40.

Europium-anti-phospho-Tyrosine Hydroxylase (Ser40) Antibody:

- TRF0204-D: 10 µg, 1,562 assay points*
- TRF0204-M: 100 µg, 15,625 assay points*

**40 fmol/assay point*

- RECOGNIZED MOTIF: RRQ**pS**LIE
- Europium-labeled rabbit polyclonal antibody recognizing phospho-Ser40 in human tyrosine hydroxylase

U*Light*™-Tyrosine Hydroxylase (Ser40) Peptide:

- TRF0111-D: 0.5 nmole, 1,000 assay points*
- TRF0111-M: 5 nmoles, 10,000 assay points*

**0.5 pmol/assay point*

- CORE SEQUENCE MOTIF: RRQ**S**LIE
  - Synthetic peptide containing the residues surrounding Ser40 of tyrosine hydroxylase
  - Phosphorylation site: Ser40

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 PAK2 kinase assay using a U*Light*-labeled peptide substrate. The binding of an Eu-labeled antibody directed against Ser40 phosphorylation of the Tyrosine Hydroxylase 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.

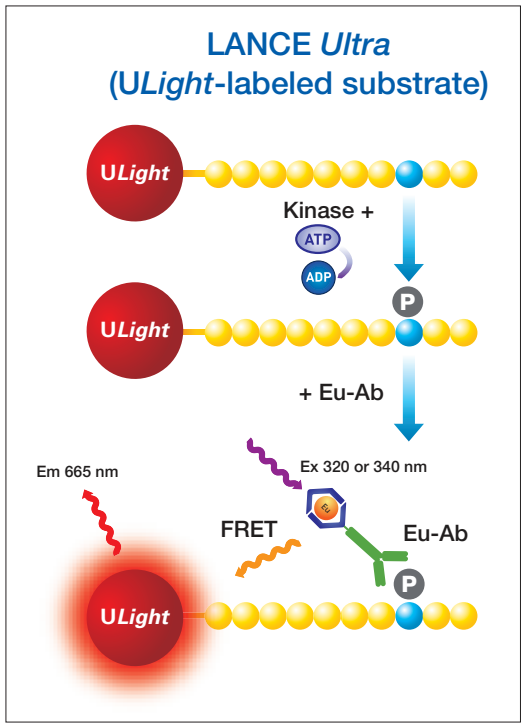

Figure 1. Schematic representation of the LANCE *Ultra* detection of a phosphorylated peptide substrate.

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Development of a PAK2 Kinase Assay:

Reagents needed for this assay:

Europium-labeled anti-phospho-Tyrosine Hydroxylase (Ser40) Antibody	PerkinElmer # TRF0204
U*Light*-Tyrosine Hydroxylase (Ser40) Peptide	PerkinElmer # TRF0111
PAK2, active	Millipore # 14-481
LANCE Detection Buffer, 10X	PerkinElmer # CR97-100
OptiPlate™-384, white	PerkinElmer # 6007299
TopSeal-A™ film	PerkinElmer # 6050195

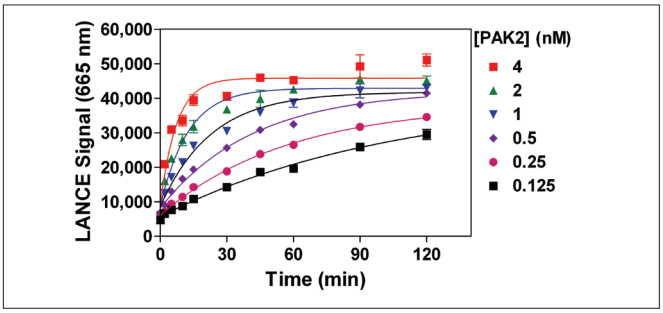
Kinase Buffer: 50 mM HEPES, pH 7.5, 1 mM EGTA, 10 mM $MgCl_2$, 2 mM DTT and 0.01% Tween-20

Standard Protocol

- Dilute kinase, ATP, inhibitors and U*Light*-Tyrosine Hydroxylase peptide in Kinase Buffer.
- Prepare a 4X Detection Mix by diluting the Eu-anti-phospho-Tyrosine Hydroxylase antibody to 8 nM in 1X LANCE Detection Buffer.
- Add to the wells of a white OptiPlate-384:
  - 5 µL of PAK2 enzyme
  - 2.5 µL of inhibitor or Kinase Buffer
  - 2.5 µL of U*Light*-Tyrosine Hydroxylase 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 the kinase reactions by adding 5 µL of 40 mM EDTA prepared in 1X LANCE Detection Buffer. Leave for 5 min at RT.
- Add 5 µL of Detection Mix (Eu-anti-phospho-Tyrosine Hydroxylase 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).

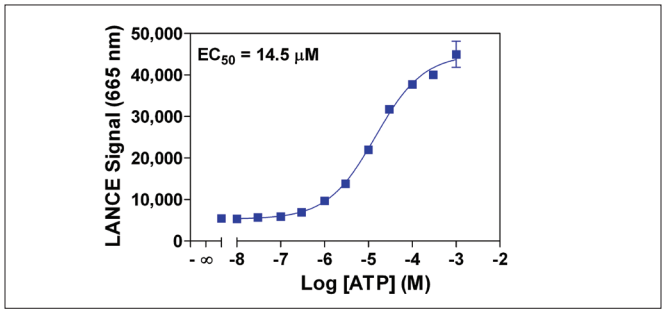
NOTE: *Eu-labeled antibodies and EDTA can be premixed just before use as a 2X concentrated Stop/Detection mix to minimize the number of liquid handling steps.*

Experiment 1: Enzymatic Titration and Time-Course



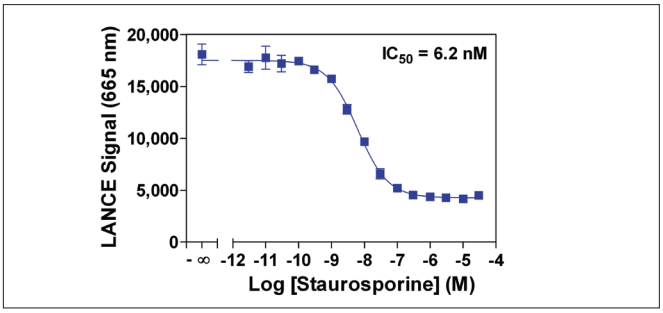
Enzymatic progress curves were produced by incubating PAK2 enzyme at concentrations ranging from 0.125 to 4 nM with 50 nM U*Light*-Tyrosine Hydroxylase peptide and 20 µM ATP. Kinase reactions were terminated at the indicated times by the addition of EDTA. Detection mix was added and signal read after 60 minutes.

Experiment 2: ATP Titration



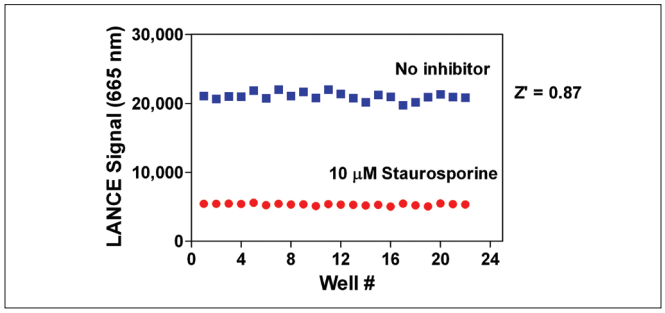
Serial dilutions of ATP ranging from 10 nM to 1 mM were added to 0.5 nM PAK2 and 50 nM of U*Light*-Tyrosine Hydroxylase peptide. Kinase reactions were terminated after 30 min by the addition of EDTA.

Experiment 3: Enzyme Inhibition



Serial dilutions of staurosporine ranging from 3 pM to 30 µM (final concentrations in 2% DMSO) were incubated with 0.5 nM PAK2, 50 nM U*Light*-Tyrosine Hydroxylase peptide and 10 µM ATP. Kinase reactions were terminated after 30 min by the addition of EDTA.

Experiment 4: Z'-factor Determination



PAK2 enzyme at 0.5 nM was incubated with 50 nM U*Light*-Tyrosine Hydroxylase peptide and 10 µM ATP with or without 10 µM staurosporine (final concentrations in 2% DMSO). Kinase reactions were terminated after 30 min by the addition of EDTA.

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