# LANCE *Ultra* PAK2 Kinase Assay

## Using U*Light*-Tyrosine Hydroxylase (Ser40) Peptide & Europium-anti-phospho-Tyrosine Hydroxylase (Ser40) Antibody

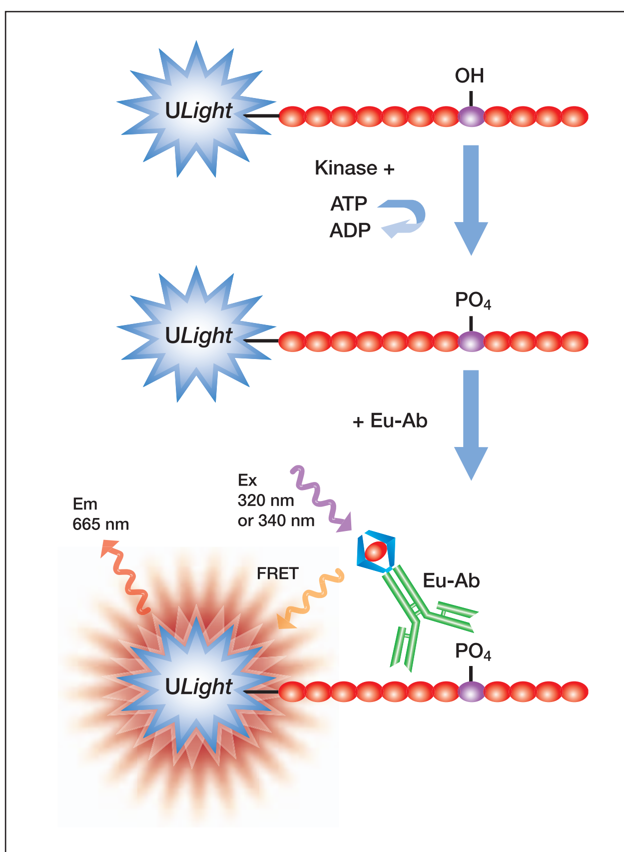


### Two LANCE® *Ultra* companion products— two convenient sizes!

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<u>S</u>LIE
  - Synthetic peptide containing the residues surrounding Ser40 of tyrosine hydroxylase
  - Phosphorylation site: Ser40
- **VALIDATED FOR KINASES:** PAK2, Aurora A, PKA, MSK1, CHK1




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<u>pS</u>LIE
- Europium-labeled rabbit polyclonal antibody recognizing phospho-Ser40 in human tyrosine hydroxylase

### 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 an 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 a PAK2 Kinase Assay

#### Additional Reagents

| | |
|---|---|
| PAK2, active | Upstate # 14-481 |
| 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



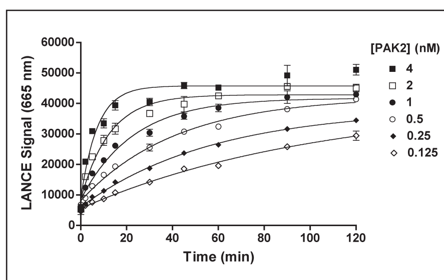
PerkinElmer® precisely.

## Suggested Procedure

- Dilute 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 and incubate at room temperature (RT).
- Stop the kinase reactions by adding 5 µL of 40 mM EDTA prepared in 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 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 and emission at 665 nm).

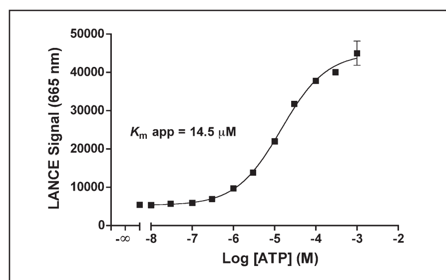
NOTE: Eu-labeled antibodies and EDTA can be premixed before use as a 2X concentrated Stop/Detection mix to minimize the number of liquid handling steps.

## Experiment 1: Enzymatic Time Course



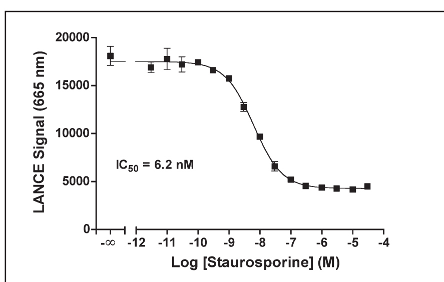

PAK2 enzyme was incubated at concentrations ranging from 0.125 to 4 nM with 50 nM U*Light*-Tyrosine Hydroxylase peptide and 20 µM ATP. Kinase reactions were terminated after 0 to 120 min by the addition of EDTA.

## Experiment 2: ATP Titration



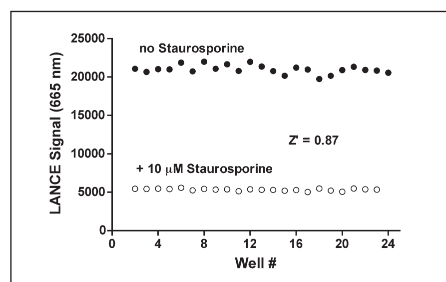

Serial dilutions of ATP ranging from 10 nM to 1 mM were added to 0.5 nM PAK2 and 50 nM of U*Light*-Tyrosine Hydroxylase peptide. Kinase reactions were terminated after 30 min by the addition of EDTA.

## Experiment 3: Enzyme Inhibition Curve




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.

## Better PAK2 Kinase Assays with a Better Technology—LANCE *Ultra*

For more information about LANCE *Ultra*, please visit www.perkinelmer.com/lanceultra or contact your local PerkinElmer Sales Representative. Learn more about our comprehensive range of reagents and consumables for drug discovery by visiting www.perkinelmer.com/drug discovery.

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