# LANCE *Ultra* p60$^{c\text{-}src}$ Kinase Assay

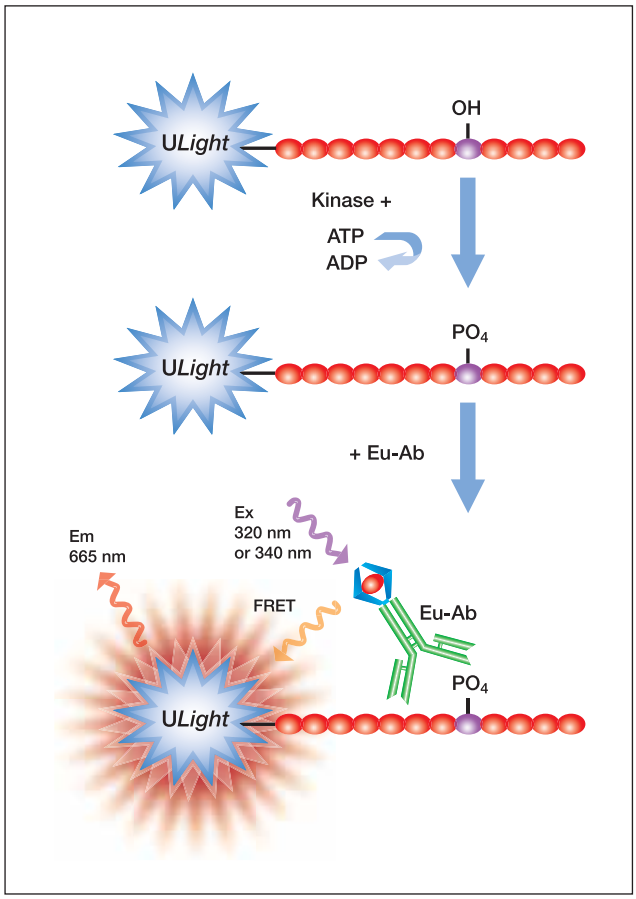
## Using U*Light*-Poly GT (4:1) & Europium-Anti-Phospho-Tyrosine (PY20) Antibody

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

U*Light*™-poly GT (4:1):

- **TRF0100-D: 1 nmole, 1,000 assay points***
- **TRF0100-M: 10 nmoles, 10,000 assay points***

  **1 pmol/assay point*
- **PEPTIDE SEQUENCE**: [Glu-**Tyr** (4:1)]$_n$
  - Generic substrate for tyrosine kinases
- **VALIDATED FOR KINASES**: p60$^{c\text{-}src}$, AXL
- **POTENTIAL SUBSTRATE FOR KINASES**: most tyrosine kinases




Europium-anti-phospho-tyrosine (PY20) antibody:

- **AD0066: 50 µg, 7,800 assay points***
- **AD0067: 1 mg, 150,000 assay points***

  **40 fmol/assay point*
- **RECOGNIZED MOTIF: pTyr**
- Mouse monoclonal IgG$_{2b}$ antibody directed against phospho-tyrosine.

### 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*, a new 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 phosphorylated U*Light*-labeled substrates brings donor and acceptor molecules into close proximity.

After irradiation of the kinase reaction at 320 nm, the energy from the Eu donor is transferred to the U*Light* acceptor 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 p60$^{c\text{-}src}$ Kinase Assay

#### Additional Reagents

| | |
|---|---|
| p60$^{c\text{-}src}$, recombinant | Upstate # 14-326 |
| LANCE Detection Buffer, 10X | PerkinElmer # CR97-100 |
| OptiPlate™-384, white | PerkinElmer # 6007299 |
| TopSeal-A™ | PerkinElmer # 6005185 |

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

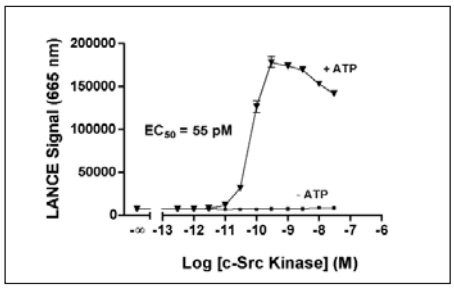
## Suggested Procedure

- Dilute kinase, ATP, inhibitors and U*Light*-poly GT (4:1) in Kinase Buffer.
- Dilute antibody (Ab) in LANCE Detection Buffer to 8 nM.
- Add to the wells of a white OptiPlate-384:
  - 5 µL of p60$^{c\text{-}src}$ enzyme,
  - 2.5 µL of inhibitor or Kinase Buffer,
  - 2.5 µL of U*Light*-poly GT (4:1)/ATP mix (for ATP titration, ATP dilutions are added separately in Kinase Buffer).
- Incubate the enzymatic reaction at room temperature (RT).
- Stop the reaction by adding 5 µL of 40 mM EDTA in Detection Buffer. Leave 5 min at RT.
- Add 5 µL of the antibody dilution (2 nM final concentration).
- 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).

## Better p60$^{c\text{-}src}$ Kinase Assays with a Better Technology—LANCE *Ultra*

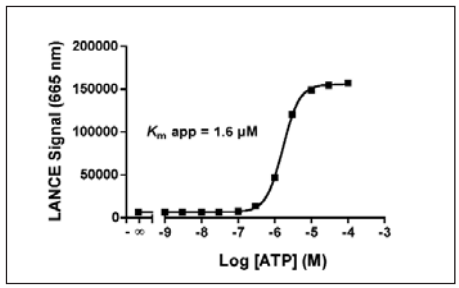
For more information about LANCE *Ultra*, please visit www.perkinelmer.com/lanceultra or contact your local PerkinElmer Sales Representative. Learn more about our comprehensive range of reagents and consumables for drug discovery by visiting www.perkinelmer.com/drugdiscovery.

## Experiment 1: Enzymatic Titration Assay



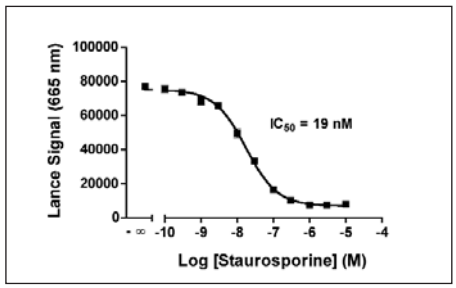

p60$^{c\text{-}src}$ enzyme at concentrations ranging from 0.3 pM to 30 nM was incubated with 100 nM U*Light*-poly GT (4:1) and 20 µM ATP. Kinase reactions were terminated after 60 min by the addition of EDTA.

## Experiment 2: ATP Titration



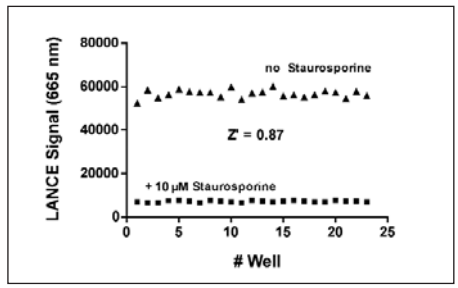

Serial dilutions of ATP ranging from 1 nM to 100 µM were added to 300 pM p60$^{c\text{-}src}$ kinase and 100 nM of U*Light*-poly GT (4:1) substrate. Kinase reactions were terminated after 60 min by the addition of EDTA.

## Experiment 3: Enzyme Inhibition Curve




Serial dilutions of staurosporine ranging from 100 pM to 10 µM (final concentrations in 2% DMSO) were pre-incubated for 5 min with the p60$^{c\text{-}src}$ enzyme (300 pM final concentration). Then 100 nM U*Light*-Poly GT (4:1) and 4 µM ATP were added. Kinase reactions were terminated after 60 min by the addition of EDTA.

## Experiment 4: Z'-factor Determination:




The p60$^{c\text{-}src}$ enzyme at 300 pM was incubated with 100 nM U*Light*-poly GT (4:1) substrate in Kinase Assay Buffer with 4 µM ATP or 10 µM staurosporine and ATP. Reactions were terminated after 60 min by the addition of EDTA.

## Complementary Products

| Product | Description | Product No. |
|---|---|---|
| Europium-anti-phospho-Tyrosine (PT66) | 50 µg<br>1 mg | AD0068<br>AD0069 |
| Europium-anti-phospho-Tyrosine (P-Tyr-100) | 10 µg<br>50 µg<br>1 mg | AD0203<br>AD0161<br>AD0162 |
| U*Light*-poly GAT, $[EA\underline{Y}(1:1:1)]_n$ | 1 nmole<br>10 nmoles | TRF0101-D<br>TRF0101-M |

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