TECH NOTE *U*-TRF #28

EphA4 Kinase Assay

KinaSelect TK Kit

New LANCE *Ultra* KinaSelect-TK kit for tyrosine kinases!

U*Light*™ -TK Peptide:

- TRF0127-D: 0.5 nmole
  1,000 assay points*
- TRF0127-M: 5 nmoles
  10,000 assay points*

**0.5 pmol/assay point*

VALIDATION:

The U*Light*-TK peptide was validated as substrate for over 85% of a panel of 83 tyrosine kinases.

Europium-anti-phosphotyrosine (PT66):

- AD0068: 50 µg
  7,800 assay points
- AD0069: 1 mg
  156,000 assay points

**40 fmol/assay point*

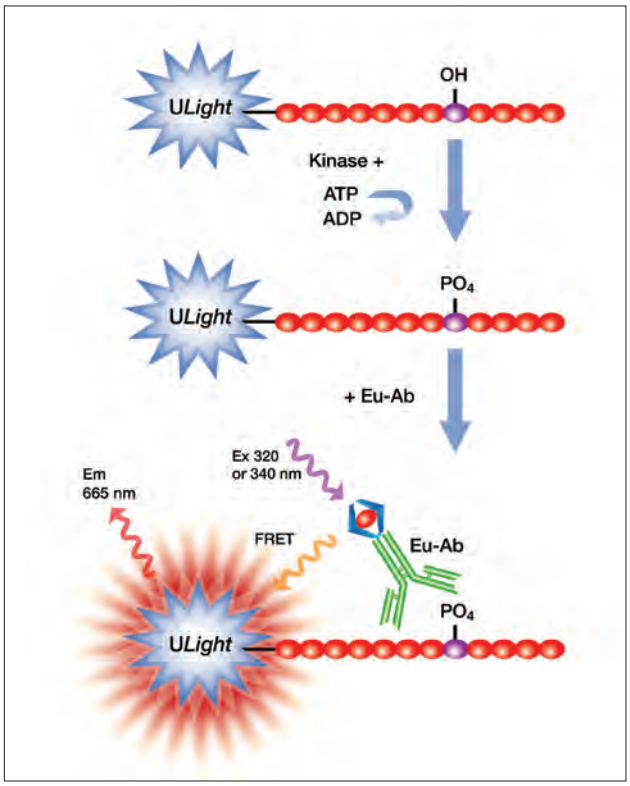
RECOGNIZED MOTIF:

Phosphorylated tyrosine residues

KinaSelect™ TK Kit

- TRF0301-D:
  1,000 assay points

Please consult the LANCE *Ultra* Selection Guide for Tyrosine Kinases at:
las.perkinelmer.com/content/Manuals/GDE_LANCEUltraTyrSelectionGuide.pdf



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 a 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 an Eph4A Kinase Assay

Additional reagents

EphA4	Carna # 08-123
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.

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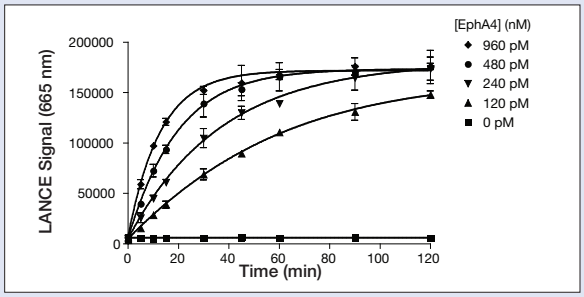
Suggested procedure

- Dilute the EphA4 enzyme, ATP, inhibitors and U*Light*-TK Peptide in Kinase Buffer.
- Prepare a 4X Detection Mix by diluting the Eu-anti-phosphotyrosine Antibody to 8 nM in 1X LANCE Detection Buffer.
- Add to the wells of a white Optiplate-384:
  - 5 µL of EphA4 enzyme
  - 2.5 µL of inhibitor or Kinase Buffer
  - 2.5 µL of U*Light*-TK 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 kinase reactions by adding 5 µL of 40 mM EDTA prepared in 1X Detection Buffer (Stop Solution). Leave for 5 min at RT.
- Add 5 µL of Detection Mix (Eu-anti-phosphotyrosine 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).

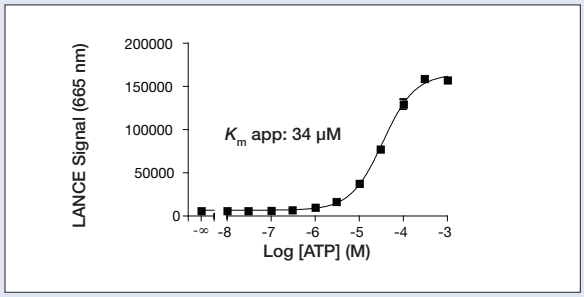
NOTE: Eu-labeled antibodies and EDTA can be premixed just before use as a 2X concentrated Stop Solution/Detection mix to minimize the number of liquid handling steps.

Experiment 1: Enzymatic Time Course



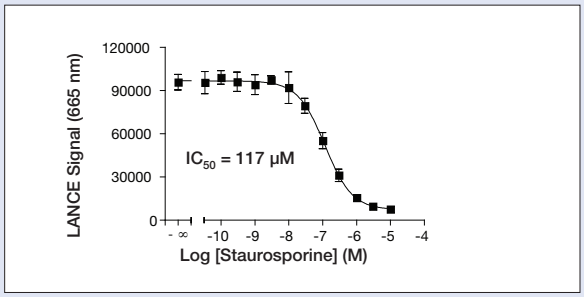
The EphA4 enzyme was incubated at concentrations ranging from 120 to 960 pM with 50 nM U*Light*-TK peptide and 200 µM ATP. Kinase reactions were terminated after 0 to 120 min by the addition of EDTA.

Experiment 2: ATP Titration



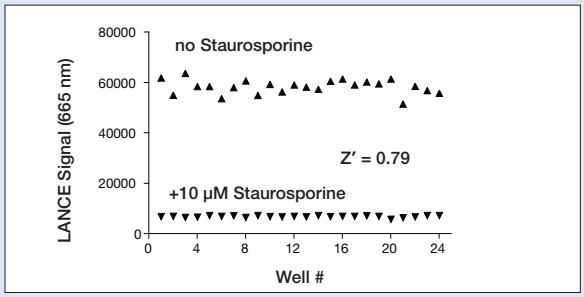
Serial dilutions of ATP ranging from 10 nM to 1 mM were incubated with 120 pM EphA4 enzyme and 50 nM of U*Light*-TK Peptide. Kinase reactions were terminated after 60 min by the addition of EDTA.

Experiment 3: Enzyme Inhibition Curve



Serial dilutions of Staurosporine ranging from 30 pM to 10 µM (final concentrations in 2% DMSO) were incubated with 120 pM EphA4 enzyme, 50 nM U*Light*-TK peptide and 35 µM ATP. Kinase reactions were terminated after 60 min by the addition of EDTA.

Experiment 4: Z'-factor Determination



EphA4 enzyme at 120 pM was incubated with 50 nM U*Light*-TK Peptide and 35 µM ATP, with or without 10 µM staurosporine (final concentrations in 2% DMSO). Kinase reactions were terminated after 60 min by the addition of EDTA.

Final comments: The KinaSelect-TK kit was designed to be a companion to the KinaSelect Ser/Thr kit to provide researchers with tools to identify optimal conditions for the study of kinase activity in the LANCE® *Ultra* assay format. All essential components are included in the kit to get started. Preliminary assays with our entire panel of U*Light* tyrosine kinase substrates have showed that the U*Light*-TK peptide substrate included in this kit provides the best performance for the receptor kinases of the EPH family in the LANCE® *Ultra* assay format (ref: TK selector guide).

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