TECH NOTE *U*-TRF #14

INSR Kinase Assay

U*Light*-IRS-1 (Tyr983) Peptide and Europium-anti-phospho-tyrosine Antibody (PT66)

Two LANCE *Ultra* companion products – two convenient sizes!

U*Light*™-IRS-1 (Tyr983) Peptide:

- TRF0120-D: 1 nmole, 1,000* assay points
- TRF0120-M: 10 nmoles, 10,000* assay points

**1 pmol/assay point*

PEPTIDE SEQUENCE:

CKKSRGD**Y**MTMQIG

Synthetic peptide derived from residues 979-989 of mouse Insulin receptor substrate 1 (IRS-1); phosphorylation site: Tyr983.

VALIDATED FOR KINASES: INSR, EphB4, FGFR1, FLT1, IGFR1, JAK1, JAK2, PDGFRa, RET, TIE2, TYK2

POTENTIAL SUBSTRATE FOR KINASES:

Tyrosine kinases specific for the YMxM motif

Europium-anti-phospho-tyrosine Antibody (PT66):

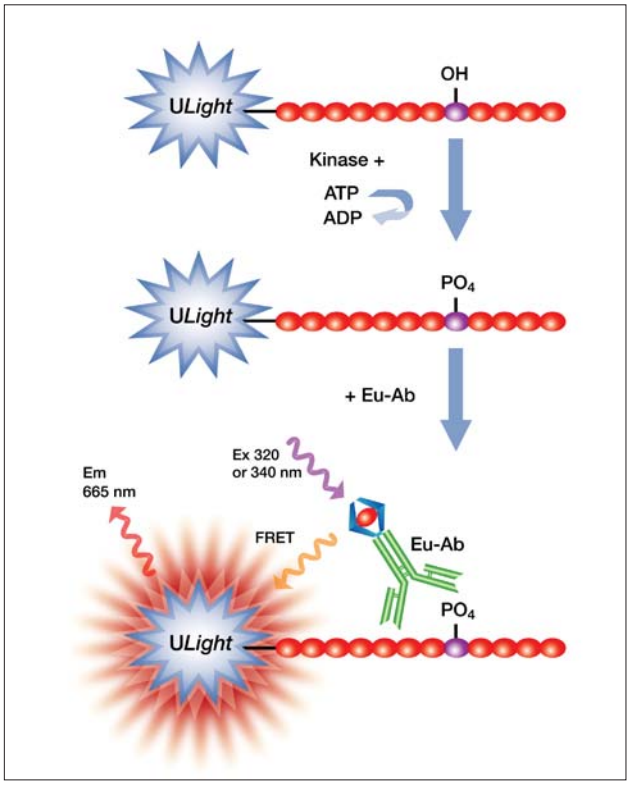
- AD0068: 50 µg, 7,500* assay points
- AD0069: 1 mg, 150,000* assay points

**40 fmol/assay point*

RECOGNIZED MOTIF:

pTyr

Mouse monoclonal 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*, 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 INSR Kinase Assay

Additional reagents

INSR active	Carna # 08-242
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.

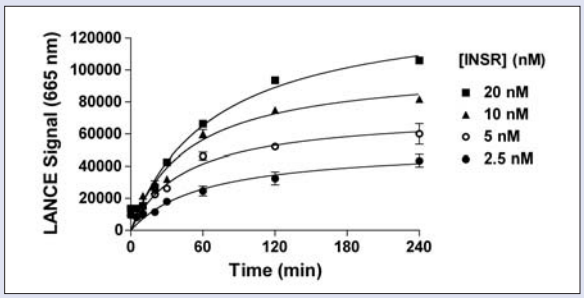
PerkinElmer® precisely.

Suggested procedure

- Dilute the INSR kinase, ATP, inhibitors and U*Light*-IRS-1 (Tyr983) peptide in Kinase Buffer.
- Prepare a 4X Detection Mix by diluting the Eu-anti-phospho-tyrosine antibody (PT66) to 8 nM in 1X LANCE Detection Buffer.
- Add to the wells of a white Optiplate-384:
  - 5 µL of INSR enzyme
  - 2.5 µL of inhibitor or Kinase Buffer
  - 2.5 µL of U*Light*-IRS-1 (Tyr983) 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 4X Detection Mix (Eu-anti-phospho–tyrosine Antibody (PT66) 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).

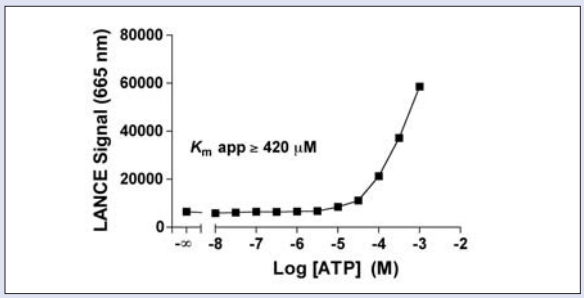
NOTE: Eu-labeled antibodies and EDTA can be premixed before use as a 2X concentrated Stop Solution/Detection Mix to minimize the number of liquid handling steps.

Experiment 1: Enzymatic Time Course



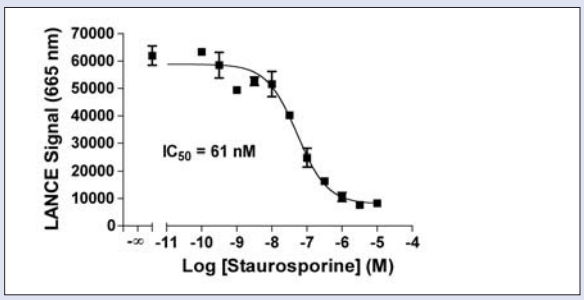
INSR enzyme was incubated at concentrations ranging from 2.5 to 20 nM with 100 nM U*Light*-IRS-1 (Tyr983) peptide and 1 mM ATP. Kinase reactions were terminated after 0 to 240 min by the addition of EDTA.

Experiment 2: ATP Titration



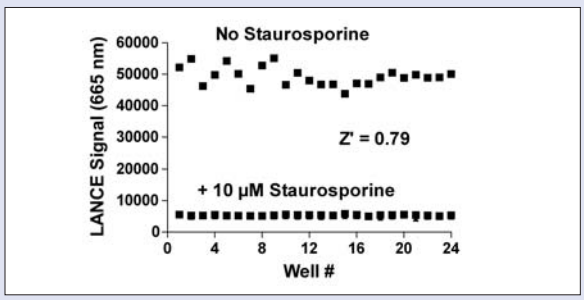
Serial dilutions of ATP ranging from 10 nM to 1 mM were added to 10 nM INSR and 100 nM U*Light*-IRS-1 (Tyr983) peptide. Kinase reactions were terminated after 4 hours by the addition of EDTA.

Experiment 3: Enzyme Inhibition Curve



Serial dilutions of staurosporine ranging from 100 pM to 10 µM (final concentrations in 1% DMSO) were incubated with 10 nM INSR, 100 nM U*Light*-IRS-1 (Tyr983) and 1 mM ATP. Kinase reactions were terminated after 4 hours by the addition of EDTA.

Experiment 4: Z'-factor Determination



INSR enzyme at 10 nM was incubated with 100 nM U*Light*-IRS-1 (Tyr983) peptide and 1 mM ATP with or without 10 µM staurosporine (final concentrations in 1% DMSO). Kinase reactions were terminated after 4 hours by the addition of EDTA.

These results show that LANCE *Ultra* reagents can be used to monitor the activity of kinases such as INSR with very high K_m for ATP.

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007870_07 Printed in USA