TECHNICAL NOTE

CK2α1/β Kinase Assay

UL*ight*™ - DNA Topoisomerase 2-alpha (Thr1342) Peptide
Europium-anti-phospho-DNA Topoisomerase
2-alpha (Thr1342) Antibody

LANCE® *Ultra*

TECH NOTE *U*-TRF #32

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

U*Light* -Topo IIα (Thr1342) Peptide:

- TRF0130-D: 0.5 nmole, 1,000 assay points*
- TRF0130-M: 5 nmoles, 10,000 assay points*

**0.5 pmol/assay point*

PEPTIDE MOTIF:

DEK**T**DDED

Synthetic peptide containing residues surrounding Thr1342 of human DNA Topoisomerase 2-alpha; phosphorylation site: Thr1342.

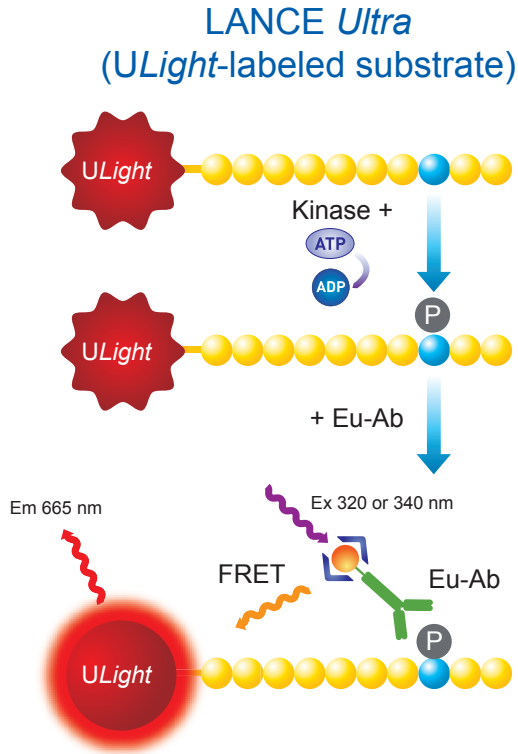
Europium-anti-phospho-Topo IIα (Thr1342) Antibody:

- TRF0218-D: 10 µg, 1,562 assay points*
- TRF0218-M: 100 µg, 15,625 assay points*

**40 fmol/assay point*

RECOGNIZED MOTIF:

Europium-labeled mouse monoclonal antibody recognizing human DNA Topoisomerase 2-alpha phosphorylated at Thr1342.



LANCE *ULTRA* KINASE ASSAYS

LANCE *Ultra* time-resolved fluorescence resonance energy transfer (TR-FRET) assays use a proprietary europium chelate donor dye 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.

PerkinElmer®
For the Better

Development of a CK2α1/β Kinase Assay

Additional Reagents:

LANCE Detection Buffer, 10X	PerkinElmer # CR97-100
OptiPlate™-384, white	PerkinElmer # 6007299
TopSeal™-A	PerkinElmer # 6005185
CK2α1/β (CSNK2A1/B)	Carna Biosciences # 05-184

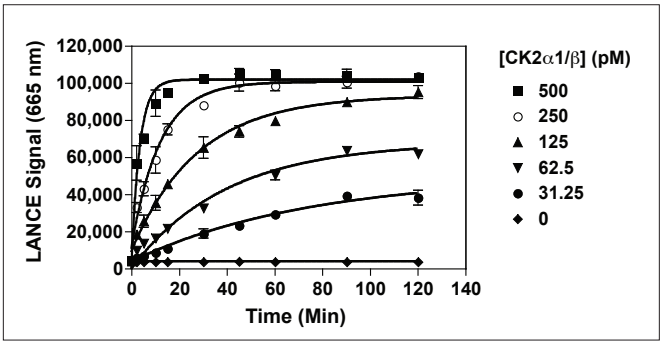
Kinase Buffer: 50 mM HEPES pH 7.5, 1 mM EGTA, 10 mM $MgCl_2$, 2 mM DTT and 0.01% Tween-20.

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.*

Suggested Procedure

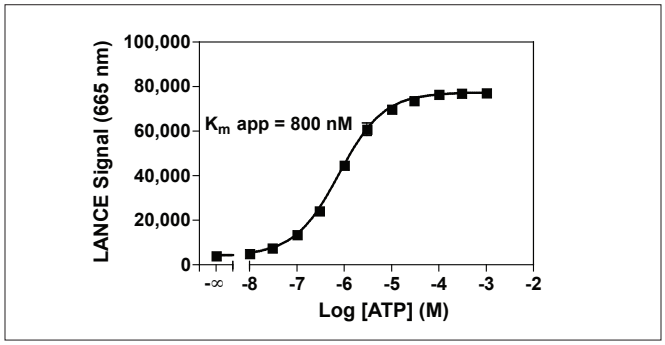
- Dilute the CK2α1/β enzyme, ATP, inhibitors and U*Light*-DNA Topoisomerase 2-alpha (Thr1342) Peptide in Kinase Buffer.
- Prepare a 4X Detection Mix by diluting the Eu-anti-phospho-DNA Topoisomerase 2-alpha (Thr1342) Antibody to 8 nM in 1X LANCE Detection Buffer.
- Add to the wells of a white Optiplate-384:
  - 5 µL of CK2α1/β enzyme
  - 2.5 µL of inhibitor or Kinase Buffer
  - 2.5 µL of U*Light*- DNA Topoisomerase 2-alpha (Thr1342) 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 24 mM EDTA prepared in 1X Detection Buffer (Stop Solution). Leave for 5 min at RT.
- Add 5 µL of Detection Mix (Eu-anti-phospho-DNA Topoisomerase 2-alpha (Thr1342) 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 & emission at 665 nm).

Experiment 1: Enzyme Titration and Time-Course



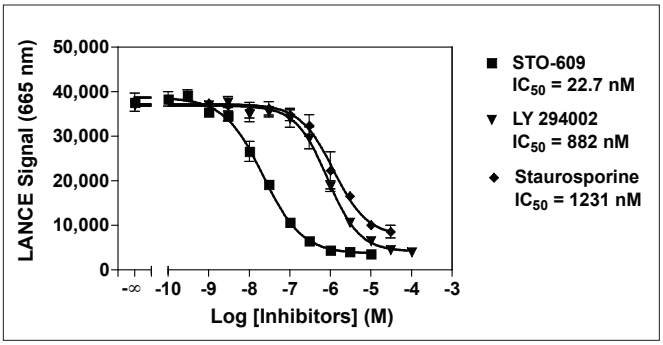
CK2α1/β enzyme was incubated at concentrations ranging from 31.25 to 500 pM with 50 nM U*Light*-Topo IIα (Thr1342) Peptide and 100 µM ATP. Kinase reactions were terminated after 0 to 120 min by the addition of EDTA.

Experiment 2: ATP Titration



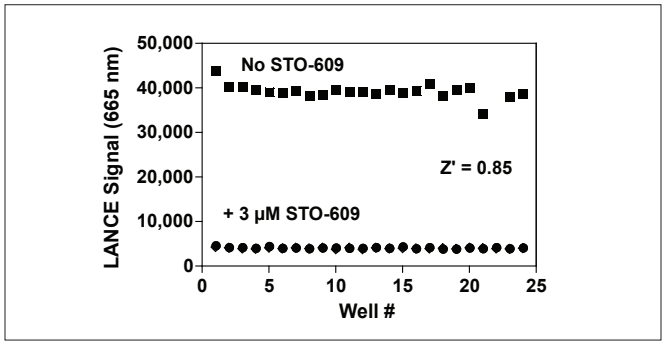
Serial dilutions of ATP ranging from 10 nM to 1 mM were added to 125 pM CK2α1/β enzyme and 50 nM of U*Light*-Topo IIα (Thr1342) Peptide. Kinase reactions were terminated after 60 min by the addition of EDTA.

Experiment 3: Enzyme Inhibition



Serial dilutions of STO-609 ranging from 100 pM to 10 µM, serial dilutions of LY 294002 ranging from 1 nM to 100 µM and serial dilutions of Staurosporine ranging from 1 nM to 30 µM (final concentrations in 2% DMSO) were incubated with 125 pM CK2α1/β enzyme, 50 nM U*Light*-Topo IIα (Thr1342) Peptide and 1 µM ATP. Kinase reactions were terminated after 60 min by the addition of EDTA.

Experiment 4: Z'-factor Determination



CK2α1/β enzyme at 125 pM was incubated with 50 nM U*Light*-Topo IIα (Thr1342) Peptide and 1 µM ATP with or without 3 µM STO-609 (final concentrations in 2% DMSO). Kinase reactions were terminated after 60 min by the addition of EDTA.

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