

PLK3 Kinase Assay

ULight[™] - DNA Topoisomerase 2-alpha (Thr1342) Peptide
Europium-anti-phospho-DNA Topoisomerase 2-alpha
(Thr1342) Antibody

LANCE[®] *Ultra*

TECH NOTE U-TRF #31

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

ULight -Topo II α (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:

DEKIDDED

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

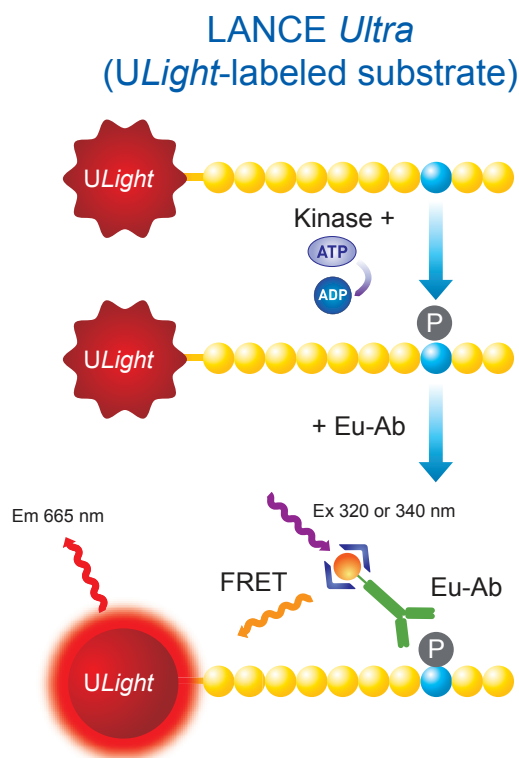
Europium-anti-phospho- Topo II α (Thr1342) Antibody:

- TRF0218-D: 10 μ g, 1,562 assay points*
- TRF0218-M: 100 μ g, 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 *ULight*, 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 *ULight*-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 *ULight* acceptor dye which, in turn, generates light at 665 nm. The intensity of the light emission is proportional to the level of *ULight*-substrate phosphorylation.

Development of a PLK3 Kinase Assay

Additional Reagents:

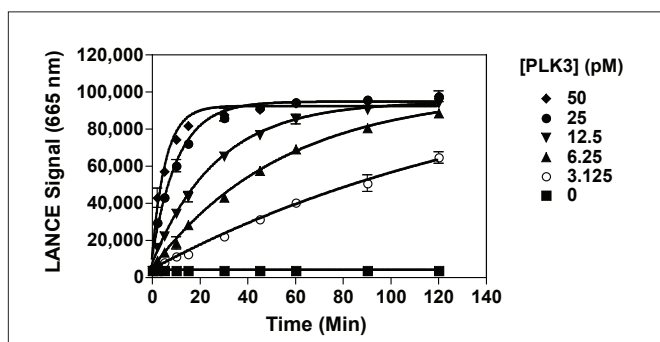
LANCE® Detection Buffer, 10X PerkinElmer # CR97-100
 OptiPlate™-384, white PerkinElmer # 6007299
 TopSeal™-A PerkinElmer # 6005185
 PLK3 Carna Biosciences # 05-159
 Kinase Buffer: 50 mM HEPES pH 7.5, 1 mM EGTA, 10 mM MgCl₂,
 2 mM DTT, 0.01% Tween-20 and 0.01% BSA.

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

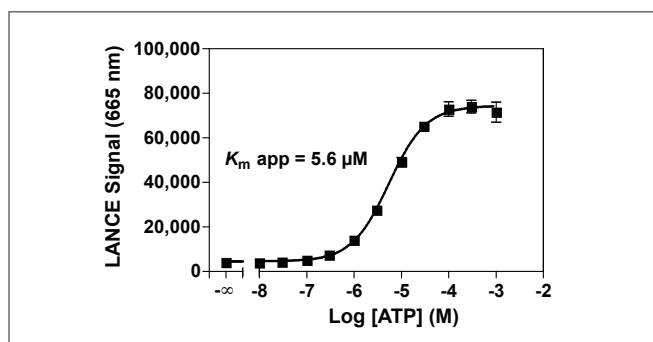
- Dilute the PLK3 enzyme, ATP, inhibitors and *ULight*-DNA Topoisomerase 2-alpha (Thr1342) Peptide in Kinase Buffer.
- Prepare a 4X Detection Mix by diluting the Eu-anti-phospho-DNA Topoisomerase 2-alpha (Thr1342) Antibody to 8 nM in 1X LANCE Detection Buffer.
- Add to the wells of a white Optiplate-384:
 - 5 µL of PLK3 enzyme
 - 2.5 µL of inhibitor or Kinase Buffer
 - 2.5 µL of *ULight*- 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- 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



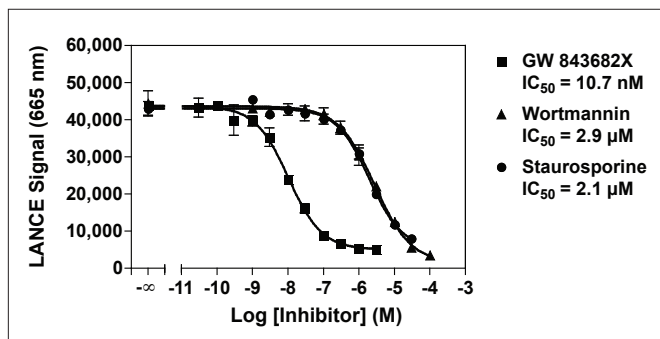
PLK3 enzyme was incubated at concentrations ranging from 3.125 to 50 pM with 50 nM *ULight*-Topo IIα (Thr1342) Peptide and 100 µM ATP. Kinase reactions were terminated after 0 to 120 min by the addition of EDTA.

Experiment 2: ATP Titration



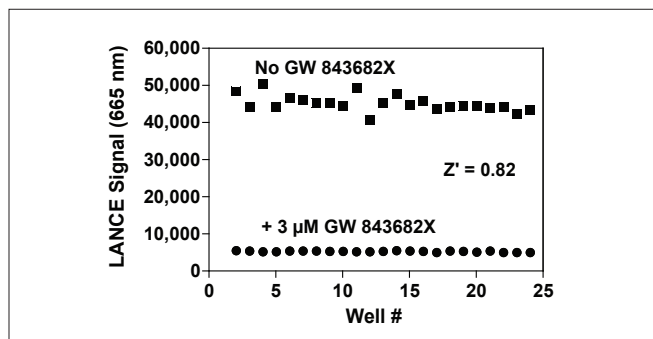
Serial dilutions of ATP ranging from 10 nM to 1 mM were added to 12.5 pM PLK3 enzyme and 50 nM of *ULight*-Topo IIα (Thr1342) Peptide. Kinase reactions were terminated after 60 min by the addition of EDTA.

Experiment 3: Enzyme Inhibition



Serial dilutions of GW 843682X ranging from 30 pM to 3 µM and serial dilutions of Staurosporine and Wortmannin ranging from 1 nM to 100 µM (final concentrations in 2% DMSO) were incubated with 12.5 pM PLK3 enzyme, 50 nM *ULight*-Topo IIα (Thr1342) Peptide and 10 µM ATP. Kinase reactions were terminated after 60 min by the addition of EDTA.

Experiment 4: Z'-factor Determination



PLK3 enzyme at 12.5 pM was incubated with 50 nM *ULight*-Topo IIα (Thr1342) Peptide and 10 µM ATP with or without 3 µM GW 843682X (final concentrations in 2% DMSO). Kinase reactions were terminated after 60 min by the addition of EDTA.

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