c-TAK1 Kinase Assay

U*Light*-Cdc25C (Ser216) Peptide and Europium-anti-phospho-(Ser) 14-3-3 binding motif 4E2 Antibody

Two LANCE *Ultra* companion products!

U*Light*™-Cdc25C (Ser216) Peptide:

- TRF0123-D: 1 nmole, 1,000* assay points
- TRF0123-M: 10 nmoles, 10,000* assay points

**1 pmol/assay point*

PEPTIDE SEQUENCE:

CSRSGLYRSP**S**MPENLNRPRL

Synthetic peptide derived from residues 207-226 of human M-phase inducer phosphatase 3 (Cdc25C); phosphorylation site: Ser216.

Europium-anti-phospho-(Ser) 14-3-3 binding motif 4E2 Antibody:

- AD0192: 10 µg, 1,562* assay points

**40 fmol/assay point*

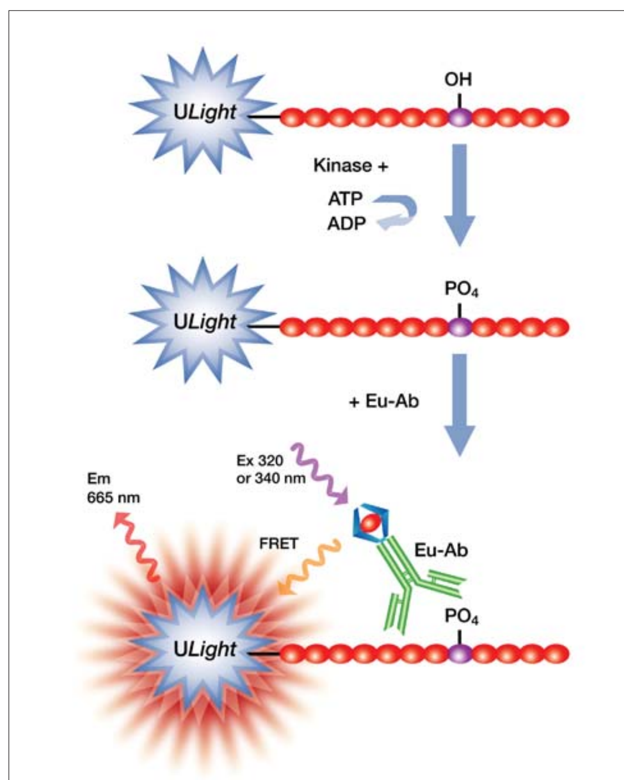
RECOGNIZED MOTIF:

(R/K)XX**pS**XP

Europium-labeled mouse monoclonal antibody.

VALIDATED FOR KINASE: c-TAK1

POTENTIAL SUBSTRATE FOR KINASES: CHK1, CHK2



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 a c-TAK1 Kinase Assay

Additional reagents

C-TAK1, active	Upstate #14-375
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.

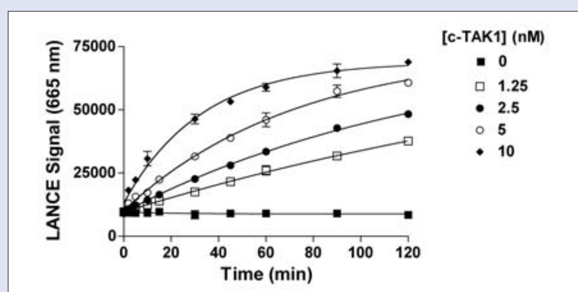
PerkinElmer®
precisely.

Suggested procedure

- Dilute the c-TAK1 kinase, ATP, inhibitors and U*Light*-Cdc25C (Ser216) peptide in Kinase Buffer.
- Prepare a 4X Detection Mix by diluting the Eu-anti-phospho (Ser) 14-3-3 binding motif 4E2 antibody to 8 nM in 1X LANCE Detection Buffer.
- Add to the wells of a white Optiplate-384:
  - 5 µL of c-TAK1 enzyme
  - 2.5 µL of inhibitor or Kinase Buffer
  - 2.5 µL of U*Light*-Cdc25C (Ser216) 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-(Ser) 14-3-3 binding motif 4E2 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).

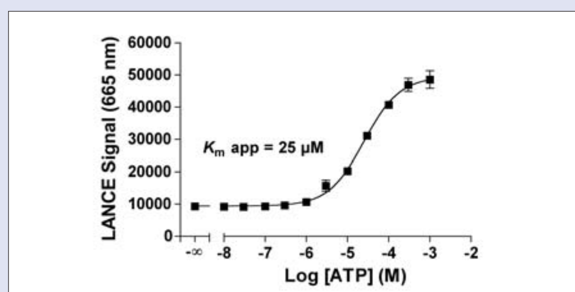
NOTE: Eu-labeled antibodies and EDTA can be premixed before use as a 2X concentrated Stop Solution/Detection Mix to minimize the number of liquid handling steps.

Experiment 1: Enzymatic Time Course



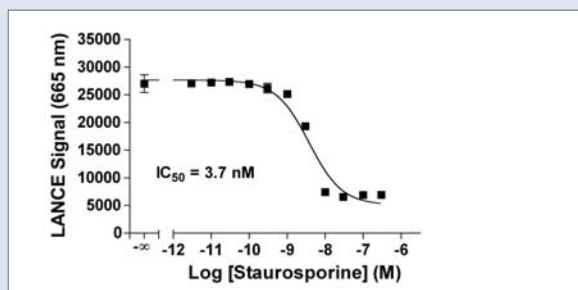
c-TAK1 enzyme was incubated at concentrations ranging from 1.25 to 10 nM with 100 nM U*Light*-Cdc25C (Ser216) peptide and 50 µM ATP. Kinase reactions were terminated after 0 to 120 min by the addition of EDTA.

Experiment 2: ATP Titration



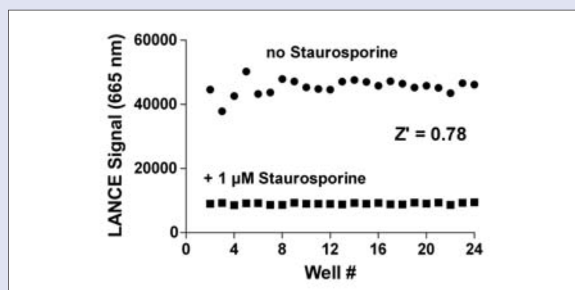
Serial dilutions of ATP ranging from 10 nM to 1 mM were added to 5 nM c-TAK1 and 100 nM U*Light*-Cdc25C (Ser216) peptide. Kinase reactions were terminated after 90 min by the addition of EDTA.

Experiment 3: Enzyme Inhibition Curve



Serial dilutions of staurosporine ranging from 3 pM to 300 nM (final concentrations in 2% DMSO) were incubated with 5 nM c-TAK1, 100 nM U*Light*-Cdc25C (Ser216) peptide and 100 µM ATP. Kinase reactions were terminated after 45 min by the addition of EDTA.

Experiment 4: Z'-factor Determination



c-TAK1 enzyme at 5 nM was incubated with 100 nM U*Light*-Cdc25C (Ser216) peptide and 100 µM ATP with or without 1 µM staurosporine (final concentrations in 2% DMSO). Kinase reactions were terminated after 45 min by the addition of EDTA.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
Phone: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer® precisely.

For a complete listing of our global offices, visit www.perkinelmer.com/lasoffices

©2007 PerkinElmer, Inc. All rights reserved. The PerkinElmer logo and design are registered trademarks of PerkinElmer, Inc. OptiPlate, TopSeal and U*Light* are trademarks and EnVision and LANCE are registered trademarks of PerkinElmer, Inc. or its subsidiaries, in the United States and other countries. All other trademarks not owned by PerkinElmer, Inc. or its subsidiaries that are depicted herein are the property of their respective owners. PerkinElmer reserves the right to change this document at any time without notice and disclaims liability for editorial, pictorial or typographical errors.

007870_04 Printed in USA