TECH NOTE *U*-TRF #19

MAPKAP Kinase 2 Assay

U*Light*-AKT/PKB (Ser473) Peptide & Europium-anti-phospho-Ser/Thr-Phe Antibody

LANCE *ULTRA*

TECHNICAL NOTE

Two LANCE *Ultra* companion products!

U*Light*™-AKT/PKB (Ser473) Peptide:

- TRF0124-D: 0.5 nmole, 1,000* assay points
- TRF0124-M: 5 nmoles, 10,000* assay points

**0.5 pmol/assay point*

PEPTIDE SEQUENCE:
CSERRPHFPQF**S**YSASGTAR

Synthetic peptide derived from residues 463-480 of human RAC-alpha serine/threonine-protein kinase (AKT1 or PKB); phosphorylation site: Ser473.

VALIDATED FOR KINASE: MAPKAP kinase 2

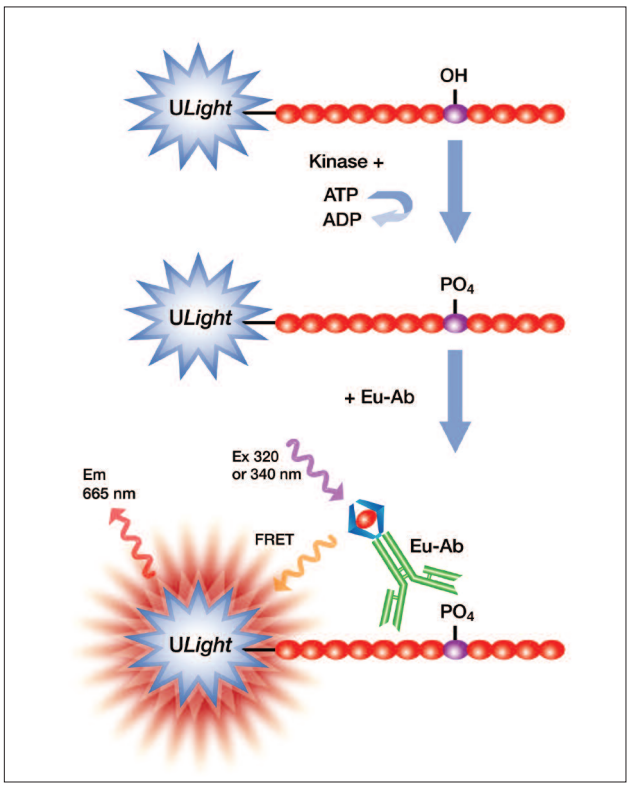
Europium-anti-phospho-Ser/Thr-Phe Antibody:

- AD0178: 10 µg, 1,562* assay points

**40 fmol/assay point*

RECOGNIZED MOTIF:
Y/F/W-**pS/pT** or **pS/pT**-F

Europium-labeled rabbit polyclonal antibody recognizing phospho-serine or threonine in the context of tyrosine, tryptophan or phenylalanine at the -1 position or phenylalanine at the +1 position.



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 MAPKAP kinase 2 Kinase Assay

Additional reagents

MAPKAP kinase 2	Upstate # 14-337
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.

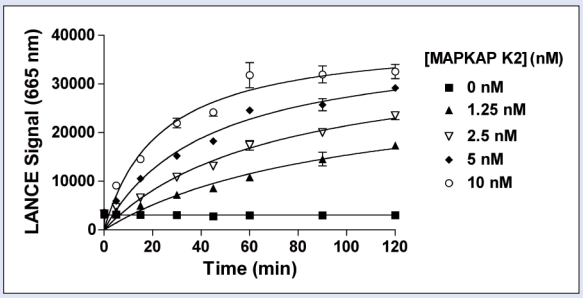
Suggested procedure

Dilute the MAPKAP kinase 2, ATP, inhibitors and U*Light*-AKT/PKB (Ser473) Peptide in Kinase Buffer.

- Prepare a 4X Detection Mix by diluting the Eu-anti-phospho Ser/Thr-Phe antibody to 8 nM in 1X LANCE Detection Buffer.
- Add to the wells of a white OptiPlate-384:
  - 5 µL of MAPKAP kinase 2 enzyme
  - 2.5 µL of inhibitor or Kinase Buffer
  - 2.5 µL of U*Light*-AKT/PKB (Ser473) Peptide/ATP mix (for ATP titration, ATP dilutions are added separately in Kinase Buffer).
- Cover the plate with TopSeal-A and incubate for 60 min 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/Thr-Phe 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).

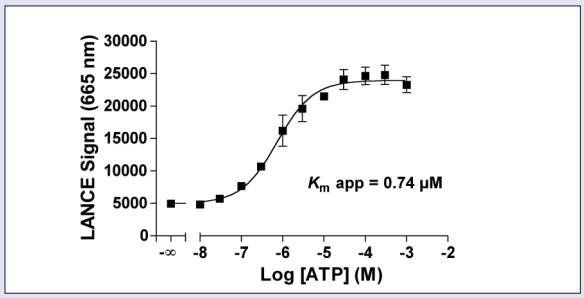
NOTE: Eu-labeled antibodies and EDTA can be premixed before use as a 2X concentrated Stop Solution/Detection mix to minimize the number of liquid handling steps.

Experiment 1: Enzymatic Time Course



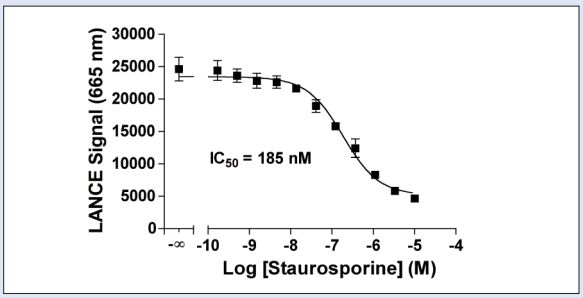
MAPKAP kinase 2 enzyme was incubated at concentrations ranging from 0 to 10 nM with 50 nM U*Light*-AKT/PKB (Ser473) Peptide and 20 µM ATP. Kinase reactions were terminated after 0 to 120 min by the addition of EDTA.

Experiment 2: ATP Titration



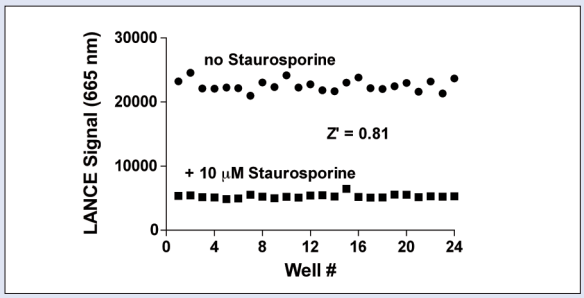
Serial dilutions of ATP ranging from 10 nM to 1 mM were added to 10 nM MAPKAP kinase 2 and 50 nM of U*Light*-AKT/PKB (Ser473) Peptide. Kinase reactions were terminated after 60 min by the addition of EDTA.

Experiment 3: Enzyme Inhibition Curve



Serial dilutions of staurosporine ranging from 3 pM to 10 µM (final concentrations in 1% DMSO) were incubated with 10 nM MAPKAP kinase 2, 50 nM U*Light*-AKT/PKB (Ser473) Peptide and 10 µM ATP. Kinase reactions were terminated after 60 min by the addition of EDTA.

Experiment 4: Z'-factor Determination



MAPKAP kinase 2 enzyme at 10 nM was incubated with 50 nM U*Light*-AKT/PKB (Ser473) Peptide and 10 µM ATP with or without 10 µM staurosporine (final concentrations in 1% DMSO). Kinase reactions were terminated after 60 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

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