TECHNICAL NOTE

MAP2K6 Kinase Assay

U*Light*™ -anti-GST & Europium-anti-phospho-MAP Kinase p38-alpha (Thr180/Tyr182) Antibody

LANCE® *Ultra*

TECH NOTE *U*-TRF #33

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Two LANCE *Ultra* companion products!

U*Light* -anti-GST:

- TRF0104-D: 1 nmole
- TRF0104-M: 10 nmoles
- TRF0104-M: 100 nmoles

Europium-anti-phospho-MAP kinase p38-alpha (Thr180/Tyr182) Antibody:

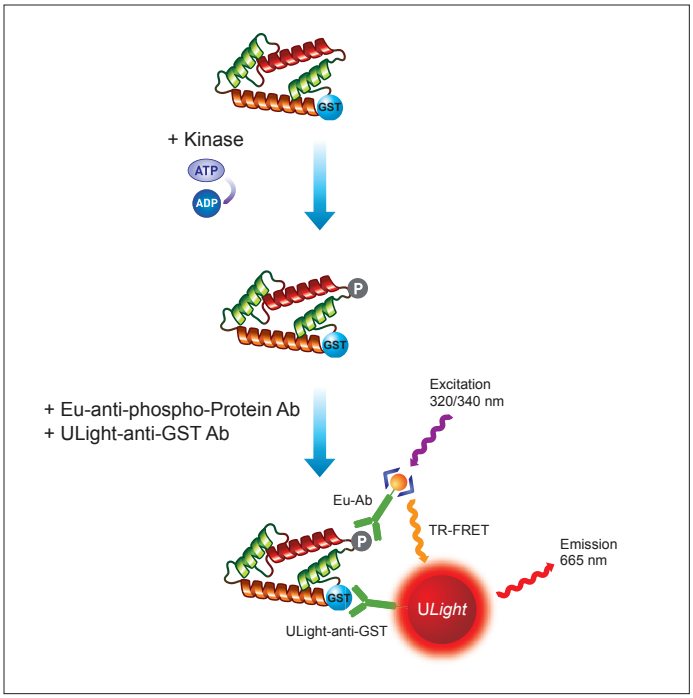
- TRF0219-D: 10 µg, 1,562 assay points*
- TRF0219-M: 100 µg, 15,625 assay points*

**40 fmol/assay point*

RECOGNIZED MOTIF:

Europium-labeled mouse monoclonal antibody recognizing human MAP kinase p38-alpha phosphorylated at Thr180 and Tyr182.

LANCE *ULTRA*
(GST-tagged substrate with U*Light*-anti-GST)



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 MAP2K6 Kinase Assay

Additional Reagents:

LANCE® Detection Buffer, 10X	PerkinElmer # CR97-100
OptiPlate™-384, white	PerkinElmer # 6007299
TopSeal™-A	PerkinElmer # 6005185
MAP2K6	Carna Biosciences #07- 046
p38α (MAPK14) inactive mutant	Carna Biosciences #04-152-11

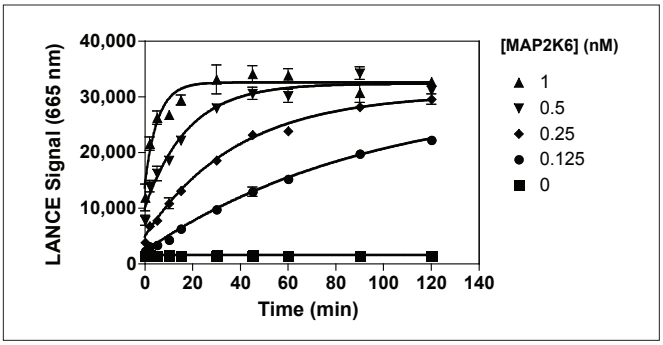
Kinase Buffer: 50 mM HEPES pH 7.5, 1 mM EGTA, 10 mM $MgCl_2$, 2 mM DTT and 0.01% Tween-20

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

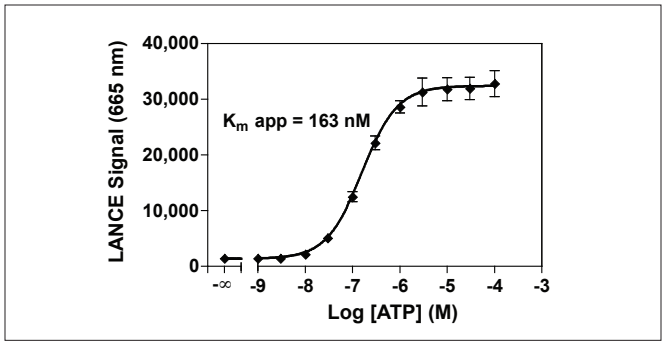
- Dilute the MAP2K6 enzyme, ATP, inhibitors and p38α (MAPK14) inactive mutant (N-terminal GST tagged) in Kinase Buffer.
- Prepare a 4X Detection Mix by diluting the Eu-anti-phospho-MAP kinase p38-alpha (Thr180/Tyr182) Antibody to 8 nM in 1X LANCE Detection Buffer.
- Add to the wells of a white Optiplate-384:
  - 5 µL of MAP2K6 enzyme
  - 2.5 µL of inhibitor or Kinase Buffer
  - 2.5 µL of p38α (MAPK14) inactive mutant (N-terminal GST tagged) / 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-MAP kinase p38-alpha (Thr180/Tyr182) Antibody at a final concentration of 2 nM and U*Light*-anti-GST at a final concentration of 30 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



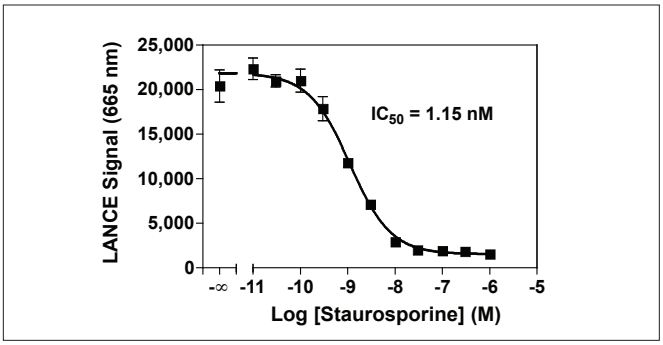
MAP2K6 enzyme was incubated at concentrations ranging from 0.125 to 1 nM with 30 nM p38α (MAPK14) inactive mutant and 100 µM ATP. Kinase reactions were terminated after 0 to 120 min by the addition of EDTA.

Experiment 2: ATP Titration



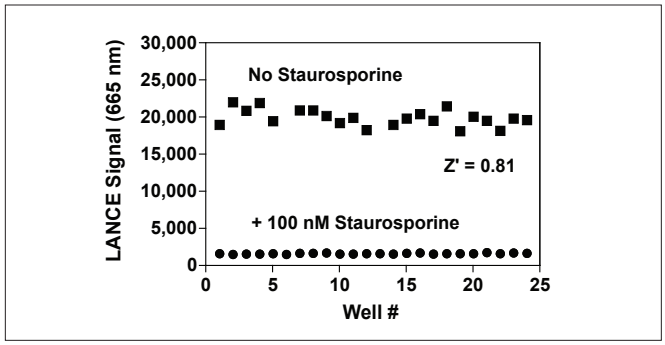
Serial dilutions of ATP ranging from 1 nM to 100 µM were added to 0.5 nM MAP2K6 enzyme and 30 nM p38α (MAPK14) inactive mutant. Kinase reactions were terminated after 45 min by the addition of EDTA.

Experiment 3: Enzyme Inhibition



Serial dilutions of Staurosporine ranging from 10 pM to 1 µM (final concentrations in 2% DMSO) were incubated with 0.5 nM MAP2K6 enzyme, 30 nM p38α (MAPK14) inactive mutant and 300 nM ATP. Kinase reactions were terminated after 45 min by the addition of EDTA.

Experiment 4: Z'-factor Determination



MAP2K6 enzyme at 0.5 nM was incubated with 30 nM p38α (MAPK14) inactive mutant and 300 nM ATP with or without 100 nM Staurosporine (final concentrations in 2% DMSO). Kinase reactions were terminated after 45 min by the addition of EDTA.

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