

LANCE *Ultra* MSK1 Kinase Assay

Using *ULight*-Crosstide Peptide & Europium-Anti-Phospho-Crosstide (GSK-3 α Ser21)

Two LANCE® *Ultra* companion products—two convenient sizes!

ULight™-Crosstide:

- **TRF0106-D: 0.5 nmole, 1,000 assay points***
- **TRF0106-M: 5 nmoles, 10,000 assay points***
*0.5 pmol/assay point
- **PEPTIDE SEQUENCE:** CGSGSGRPRTSSFAEG
 - Synthetic peptide derived from glycogen synthase kinase-3 alpha (GSK-3 α)
 - Phosphorylation site: Ser21
- **VALIDATED FOR KINASES:** PAK2, IKK β , AKT1, PKA, MSK1, MAPKAP-K1 (p90^{RSK} or S6K)
- **POTENTIAL SUBSTRATE FOR KINASES:** MAPKAP-K2, SMP-1, STK 31, TAOK-3, AKT2, AKT3, JIK

Europium-anti-phospho-Crosstide (GSK-3 α Ser21):

- **TRF0202-D: 10 μ g, 1,562 assay points***
- **TRF0202-M: 100 μ g, 15,625 assay points***
*40 fmol/assay point
- **RECOGNIZED MOTIF:** GSGSGRPRTSpSFAEG
- Europium-labeled mouse monoclonal antibody recognizing phospho-Ser21 in peptides derived from glycogen synthase kinase-3 alpha (GSK-3 α)

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 *ULight*, a new 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 phosphorylated *ULight*-labeled substrates brings donor and acceptor molecules into close proximity.

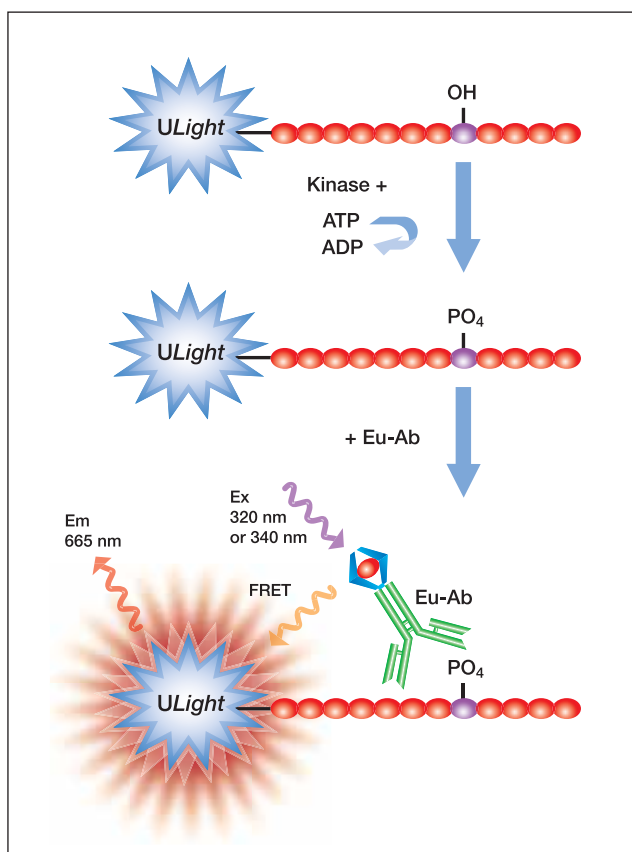
After irradiation of the kinase reaction at 320 nm, the energy from the Eu donor is transferred to the *ULight* acceptor which, in turn, generates light at 665 nm. The intensity of the light emission is proportional to the level of *ULight*-substrate phosphorylation.

Development of a MSK1 Kinase Assay

Additional Reagents

MSK1, active	Upstate # 14-548
LANCE Detection Buffer, 10X	PerkinElmer # CR97-100
OptiPlate™-384, white	PerkinElmer # 6007299
TopSeal-A™	PerkinElmer # 6005185

Kinase Buffer: 50 mM Tris-HCl, pH 7.5, 1 mM EGTA, 10 mM MgCl₂, 2 mM DTT and 0.01% Tween-20



Suggested Procedure

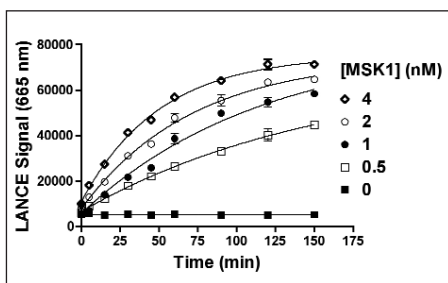
- Dilute kinase, ATP, inhibitors and *ULight*-Crosstide in Kinase Buffer.
- Dilute antibody (Ab) in LANCE Detection Buffer to 8 nM.
- Add to the wells of a white OptiPlate-384:
 - 5 μL of MSK1 enzyme,
 - 2.5 μL of inhibitor or Kinase Buffer,
 - 2.5 μL of *ULight*-Crosstide/ATP mix (for ATP titration, ATP dilutions are added separately in Kinase Buffer).
- Incubate enzymatic reactions at room temperature (RT).
- Stop the reaction by adding 5 μL of 40 mM EDTA in Detection Buffer. Leave 5 min at RT.
- Add 5 μL of the antibody dilution (2 nM final concentration).
- 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).

Better MSK1 Kinase Assays with a Better Technology — LANCE Ultra

For more information about LANCE *Ultra*, please visit www.perkinelmer.com/lanceultra or contact your local PerkinElmer Sales Representative. Learn more about our comprehensive range of reagents and consumables for drug discovery by visiting www.perkinelmer.com/drugdiscovery.

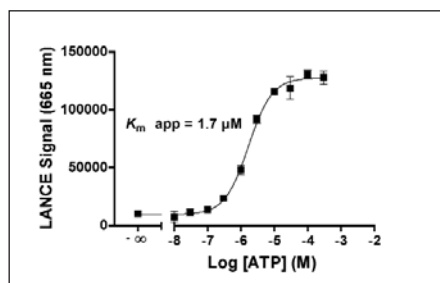
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Experiment 1: Enzymatic Time Course



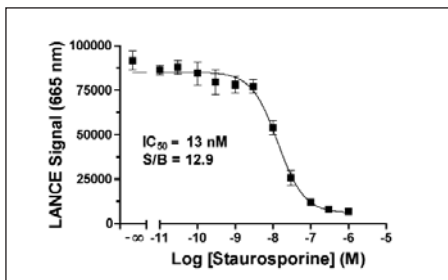
MSK1 enzyme at concentrations ranging from 0.5 to 4 nM was incubated with 50 nM *ULight*-Crosstide and 3 μM ATP. Kinase reactions were terminated after 0 to 150 min by the addition of EDTA.

Experiment 2: ATP Titration



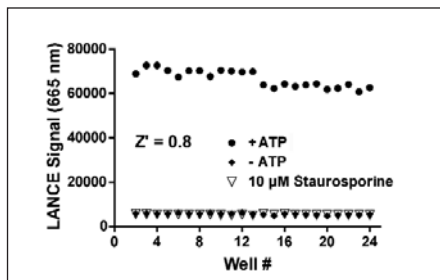
Serial dilutions of ATP ranging from 10 nM to 300 μM were added to 2 nM of MSK1 kinase and 50 nM of *ULight*-Crosstide substrate. Kinase reactions were terminated after 60 min by the addition of EDTA.

Experiment 3: Enzyme Inhibition Curve



Serial dilutions of staurosporine ranging from 100 nM to 1 μM (final concentrations in 2% DMSO) were pre-incubated for 5 min with the MSK1 enzyme (2 nM final concentration). Then 50 nM *ULight*-Crosstide and 3 μM ATP were added. Kinase reactions were terminated after 120 min by the addition of EDTA.

Experiment 4: Z'-factor Determination



The MSK1 enzyme at 2 nM was incubated with 50 nM *ULight*-Crosstide substrate in Kinase Assay Buffer with 3 μM ATP, 10 μM staurosporine and ATP, or without ATP. 2% DMSO was included. Reactions were terminated after 120 min by the addition of EDTA.

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