LANCE *Ultra* ERK1 Assay

Using U*Light*-Myelin Basic Protein (MBP) Peptide & Europium-Anti-Phospho-MBP Peptide Antibody

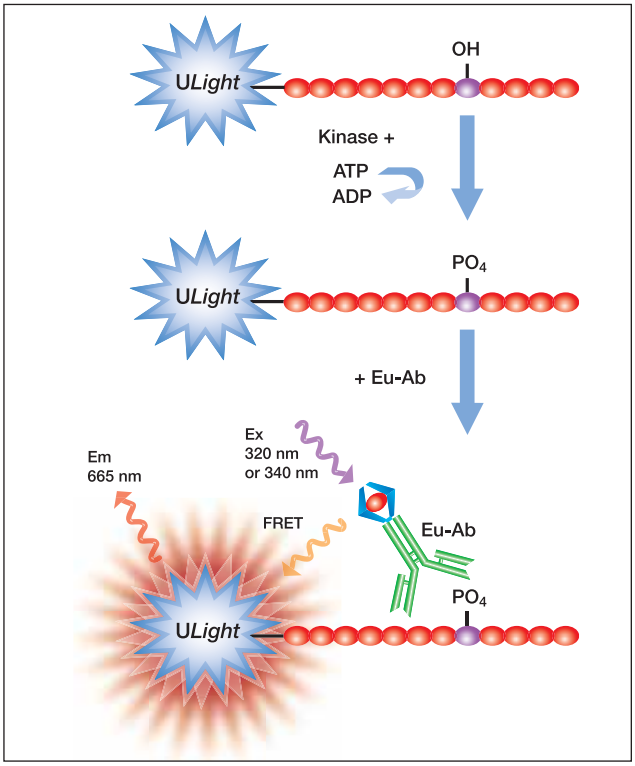
Two LANCE® *Ultra* companion products—two convenient sizes!

U*Light*™-MBP Peptide:

- **TRF0109-D: 0.5 nmole, 1,000 assay points***
- **TRF0109-M: 5 nmoles, 10,000 assay points***

  **0.5 pmol/assay point*
- **PEPTIDE SEQUENCE**: CFFKNIVTPR**T**PPPSQGK-amide
  - Synthetic peptide derived from human myelin basic protein (MBP)
  - Phosphorylation site: THR232*

  **corresponds to Thr98 in other MBP isoforms or species*
- **VALIDATED FOR KINASES**: ERK1, ERK2, CDK3/CycE
- **POTENTIAL SUBSTRATE FOR KINASES**: s6K, p43, p38



Europium-anti-phospho-Myelin Basic Protein (Thr232) antibody:

- **TRF0201-D: 10 µg, 1,562 assay points***
- **TRF0201-M: 100 µg, 15,625 assay points***

  **40 fmol/assay point*
- **RECOGNIZED MOTIF**: FFKNIVTPR**pT**PPPSQGK
- Europium-labeled mouse monoclonal antibody recognizing phospho-Thr232 in human Myelin Basic Protein (Swiss–Prot: P02686)

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*, 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 U*Light*-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 U*Light* acceptor 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 ERK1 Kinase Assay

Additional Reagents

ERK1, active	Upstate # 14-439
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 $MgCl_2$, 2 mM DTT and 0.01% Tween-20

PerkinElmer® precisely.

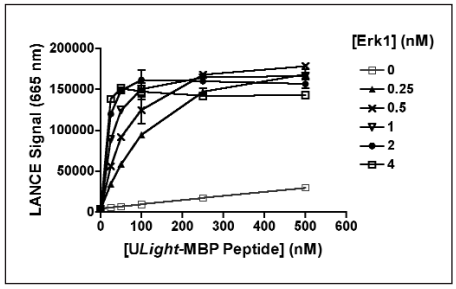
Suggested Procedure

- Dilute kinase, ATP, inhibitors and U*Light*-MBP 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 ERK1 enzyme,
  - 2.5 µL of inhibitor or Kinase Buffer,
  - 2.5 µL of U*Light*-MBP/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 ERK1 Kinase Assays with a Better Technology—LANCE *Ultra*

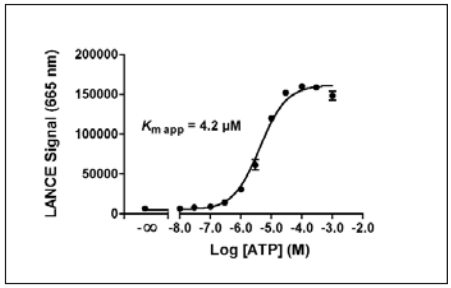
For more information about LANCE *Ultra*, please visit www.perkinelmer.com/lanceultra or contact your local PerkinElmer Sales Representative. Learn more about our comprehensive range of reagents and consumables for drug discovery by visiting www.perkinelmer.com/drug discovery.

Experiment 1: Enzymatic Time Course



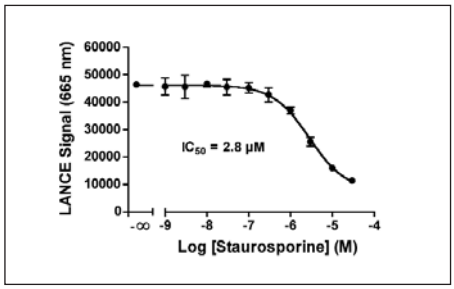
ERK1 enzyme ranging from 0.25 to 4 nM was incubated with the U*Light*-MBP substrate ranging from 25 to 500 nM in kinase assay buffer supplemented with 20 µM ATP. Reactions were terminated after 90 min by the addition of EDTA.

Experiment 2: ATP Titration



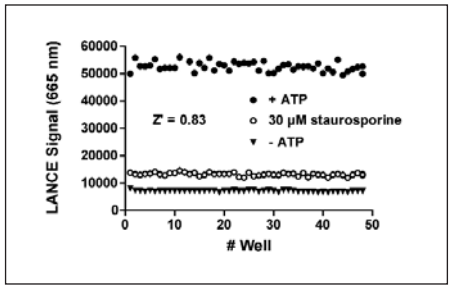
Serial dilutions of ATP ranging from 10 nM to 1 µM were added to 1 nM of ERK1 kinase and 50 nM of U*Light*-MBP substrate. Kinase reactions were terminated after 90 min by the addition of EDTA.

Experiment 3: Enzyme Inhibition Curve



Serial dilutions of staurosporine ranging from 1 nM to 30 µM (final concentrations in 2% DMSO) were pre-incubated for 5 min with the ERK1 enzyme (1 nM final concentration). Then 50 nM U*Light*-MBP and 4 µM ATP were added. Kinase reactions were terminated after 60 min by the addition of EDTA.

Experiment 4: Z'-factor Determination



The ERK1 enzyme at 1 nM was incubated with 50 nM U*Light*-MBP substrate in kinase assay buffer with 4 µM ATP, 30 µM staurosporine and ATP, or without ATP. 2% DMSO was included. Reactions were terminated after 60 min by the addition of EDTA.

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007755_08 Printed in USA