LANCE *Ultra* PKA Kinase Assay

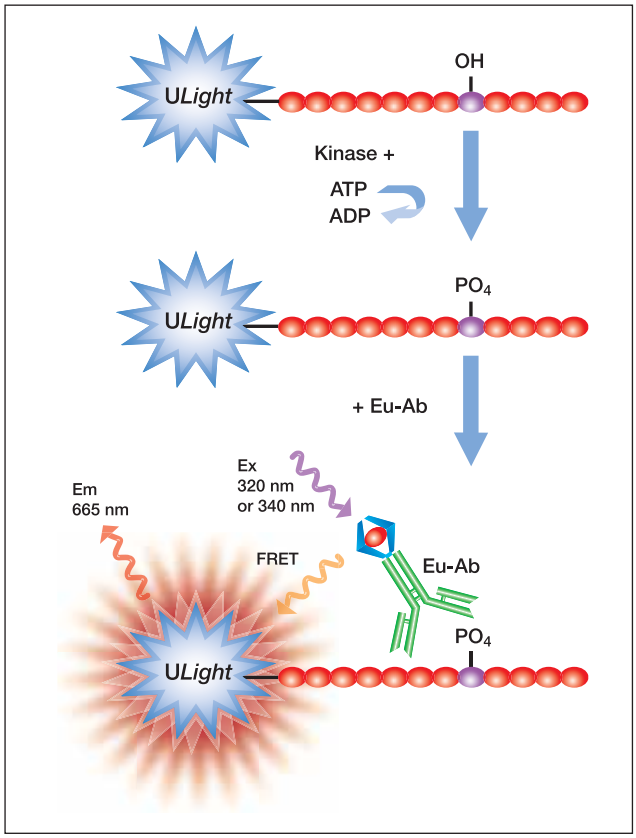
Using U*Light*-CREBtide Peptide & Europium-Anti-Phospho-CREB Antibody

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

U*Light*™-CREBtide peptide:

- **TRF0107-D: 0.5 nmole, 1,000 assay points***
- **TRF0107-M: 5 nmoles, 10,000 assay points***

  **0.5 pmol/assay point*
- **PEPTIDE SEQUENCE**: CKRREILSRRP**S**YRK
  - Synthetic peptide derived from human cAMP Response Element Binding (CREB) protein
  - Phosphorylation site: Ser133
- **VALIDATED FOR KINASES**: PKA, PKCα, IRAK4, Aurora A, PAK2, IKKβ, Akt1, PKA, MSK1, MAPKAP-K1
- **POTENTIAL SUBSTRATE FOR KINASES**: Aurora B, Aurora C, GSK-3, MSK2, PDK-2, PKG, MAPKAP-K2 and others



Europium-anti-phospho-CREB (Ser133):

- **TRF0200-D: 10 µg, 1,562 assay points***
- **TRF0200-M: 100 µg, 15,625 assay points***

  **40 fmol/assay point*
- **RECOGNIZED MOTIF**: KRREILSRRP**pS**YRK
- Mouse monoclonal antibody $IgG_{1\kappa}$ directed against phosphorylated Ser133 from human cAMP Response Element Binding (CREB) protein

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 PKA Kinase Assay

Additional Reagents

PKA, catalytic subunit, recombinant	Upstate # 14-440
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.

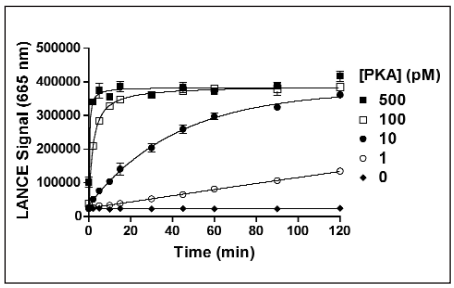
Suggested Procedure

- Dilute kinase, ATP, inhibitors and U*Light*-CREBtide 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 PKA enzyme,
  - 2.5 µL of inhibitor or Kinase Buffer,
  - 2.5 µL of U*Light*-CREBtide/ATP mix (for ATP titration, ATP dilutions are added separately in Kinase Buffer).
- Incubate the enzymatic reaction 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 PKA Kinase Assays with a Better Technology—LANCE *Ultra*

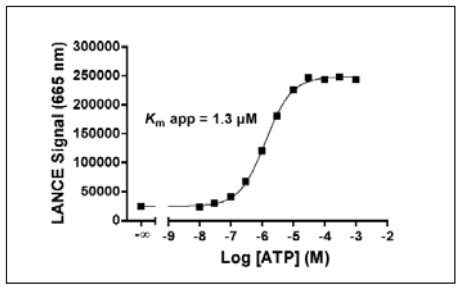
For more information about LANCE *Ultra*, please visit www.perkinelmer.com/lanceultra or contact your local PerkinElmer Sales Representative. Learn more about our comprehensive range of reagents and consumables for drug discovery by visiting www.perkinelmer.com/drug discovery.

Experiment 1: Enzymatic Time Course



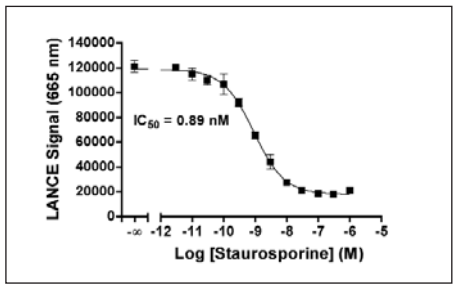
PKA enzyme at concentrations ranging from 1 to 500 pM was incubated with 50 nM U*Light*-CREBtide and 20 µM ATP. Kinase reactions were terminated after 0 to 120 min by the addition of EDTA.

Experiment 2: ATP Titration



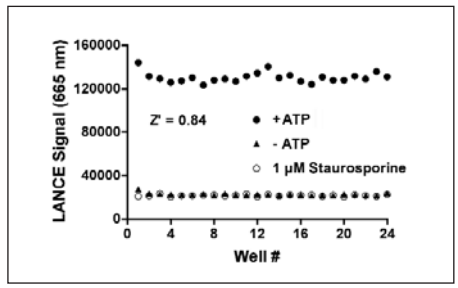
Serial dilutions of ATP ranging from 10 nM to 1 mM were added to 10 nM PKA kinase and 50 nM of U*Light*-CREBtide substrate. Kinase reactions were terminated after 60 min by the addition of EDTA.

Experiment 3: Enzyme Inhibition Curve



Serial dilutions of staurosporine ranging from 3 pM to 1 µM (final concentrations in 2% DMSO) were pre-incubated for 5 min with the PKA enzyme (10 pM final concentration). Then 50 nM U*Light*-CREBtide and 1.5 µM ATP were added. Kinase reactions were terminated after 60 min by the addition of EDTA.

Experiment 4: Z'-factor Determination



The PKA enzyme at 10 pM was incubated with 50 nM U*Light*-CREBtide substrate in Kinase Assay Buffer with 1.5 µM ATP, 1 µM staurosporine and ATP, or without ATP. Reactions were terminated after 60 min by the addition of EDTA.

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