LANCE *Ultra* AMPKα1 kinase assay

This LANCE *Ultra* kinase assay measures the phosphorylation of an Acetyl-CoA Carboxylase peptide substrate at Ser79.

Europium-anti-phospho-acetyl-CoA carboxylase (Ser79) antibody:

- TRF0208-D: 10 µg, 1,562* assay points
- TRF0208-M: 100 µg, 15,625* assay points

**40 fmol/assay point*

U*Light*™-acetyl-CoA Carboxylase (Ser79) peptide (SAMS peptide):

- TRF0118-D: 0.5 nmole, 1,000 assay points*
- TRF0118-M: 5 nmoles, 10,000 assay points*

**0.5 pmol/assay point*

Potential substrate for kinases

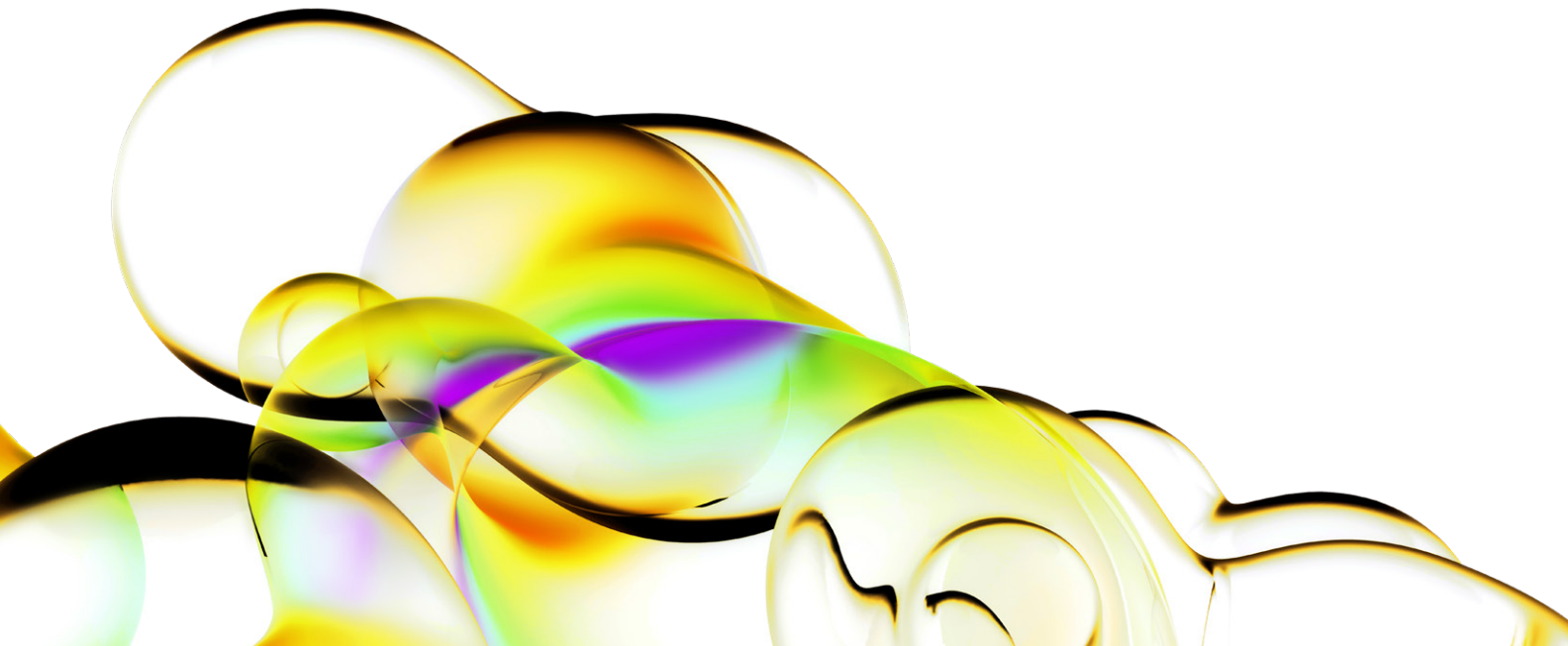
AMP-activated subfamily of protein kinases

Recognized motif:

SSM**pS**GL

Europium-labeled mouse monoclonal antibody recognizing phospho-Ser79 of rat acetyl-CoA carboxylase.

For research purposes only.
Not for use in diagnostic procedures.



Peptide sequence:

CHMRSAM**S**GLHLVKRR

Synthetic peptide derived from residues 73-85 of rat acetyl-CoA carboxylase in which Ser77 is mutated to Ala; phosphorylation site: Ser79.

LANCE *Ultra* kinase assays:

LANCE *Ultra* time-resolved fluorescence resonance energy transfer (TR-FRET) assays use a proprietary europium chelate donor dye, W1024 (Eu), together with U*Light*™, a small molecular weight acceptor dye with a red-shifted fluorescent emission.

In this technical note, we present the optimization of a AMPKalpha1 kinase assay using a U*Light*-labeled peptide substrate. The binding of a Eu-labeled antibody directed against Ser79 phosphorylation of the SAMS peptide substrate brings the Eu donor and U*Light* acceptor dye molecules into close proximity. Upon irradiation 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.

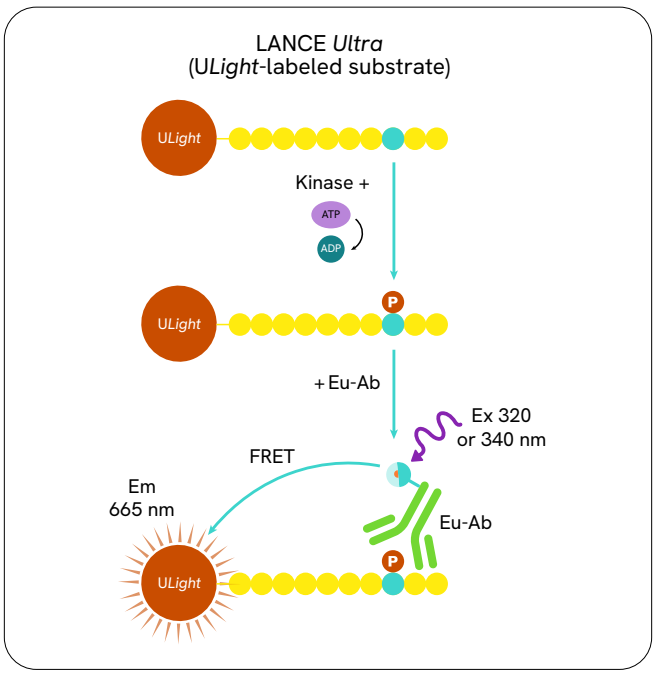


Figure 1: Schematic representation of the LANCE *Ultra* detection of a phosphorylated peptide substrate.

Development of an AMPKα1 kinase assay

Reagents needed for this assay:

Europium-labeled anti-phospho-Acetyl-CoA Carboxylase (Ser79) Antibody	Revvity # TRF0208
U*Light*-Acetyl-CoA Carboxylase (Ser79) Peptide	Revvity # TRF0118
AMPKa1, active	Carna # 02-113
LANCE® Detection Buffer, 10X	Revvity # CR97-100
OptiPlate™-384, white	Revvity # 6007299
TopSeal™-A film	Revvity # 6050195

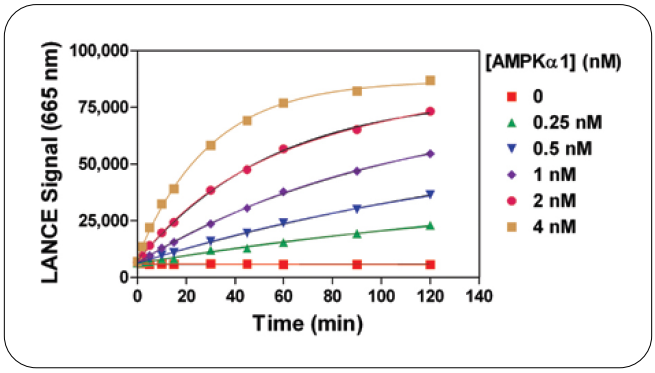
Kinase Buffer: 50 mM HEPES pH 7.5, 1 mM EGTA, 10 mM $MgCl_2$, 2 mM DTT and 0.01% Tween-20.

Standard protocol

- Dilute the AMPKα1 kinase, ATP, inhibitors and U*Light*-Acetyl-CoA Carboxylase peptide in Kinase Buffer.
- Prepare a 4X Detection Mix by diluting the Eu-anti-phospho-Acetyl-CoA Carboxylase antibody to 8 nM in 1X LANCE Detection Buffer.
- Add to the wells of a white OptiPlate-384:
  - 5 µL of AMPKα1 enzyme
  - 2.5 µL of inhibitor or Kinase Buffer
  - 2.5 µL of U*Light*-Acetyl-CoA Carboxylase peptide/ATP mix (for ATP titration, ATP dilutions are added separately in Kinase Buffer).
- Cover the plate with TopSeal-A film and incubate at room temperature (RT).
- Stop kinase reactions by adding 5 µL of 40 mM EDTA prepared in 1X LANCE Detection Buffer (Stop Solution). Leave for 5 min at RT.
- Add 5 µL of 4X Detection Mix (Eu-anti-phospho-Acetyl-CoA Carboxylase antibody at a final concentration of 2 nM).
- Cover with TopSeal-A film and incubate for 1 h at RT.
- Remove the TopSeal-A film and read signal with the EnVision® Multilabel Reader in TR-FRET mode (excitation at 320 or 340 nM and emission at 665 nM).

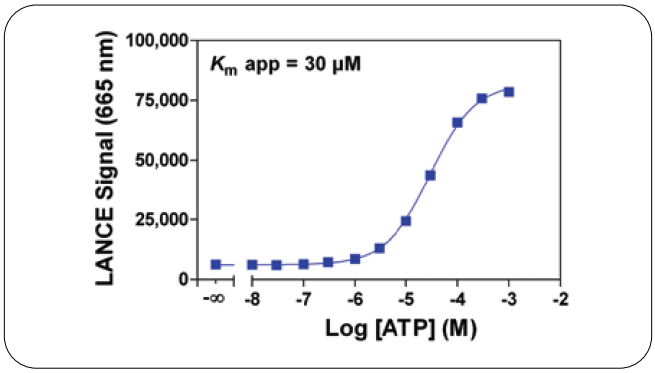
Note: Eu-labeled antibodies and EDTA can be premixed just before use as a 2X concentrated Stop Solution/Detection Mix to minimize the number of liquid handling steps.

Experiment 1: Enzymatic titration and time course



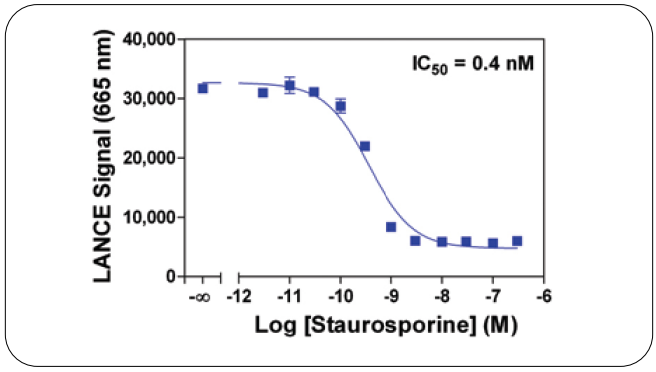
Enzymatic progress curves were produced by incubating AMPKα1 enzyme at concentrations ranging from 0.25 to 4 nM with 50 nM U*Light*-Acetyl-CoA Carboxylase peptide and 20 µM ATP. Kinase reactions were terminated at the indicated times by the addition of EDTA. Detection mix was added and signal read after 60 minutes.

Experiment 2: ATP titration



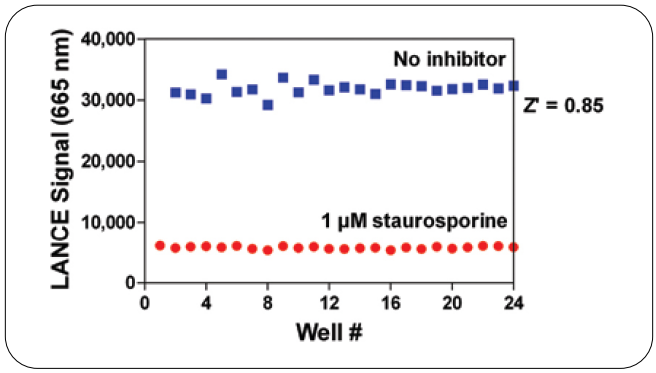
Serial dilutions of ATP ranging from 10 nM to 1 mM were added to 2 nM AMPKα1 and 50 nM U*Light*-Acetyl-CoA Carboxylase peptide. Kinase reactions were terminated after 30 min by the addition of EDTA.

Experiment 3: Enzyme inhibition curve



Serial dilutions of staurosporine ranging from 3 pM to 300 nM (final concentrations in 2% DMSO) were incubated with 2 nM AMPKα1, 50 nM U*Light*-Acetyl-CoA Carboxylase peptide and 30 µM ATP. Kinase reactions were terminated after 30 min by the addition of EDTA.

Experiment 4: Z′-factor determination



AMPKα1 enzyme at 2 nM was incubated with 50 nM U*Light*-Acetyl-CoA Carboxylase peptide and 30 µM ATP, with or without 1 µM staurosporine (final concentrations in 2% DMSO). Kinase reactions were terminated after 30 min by the addition of EDTA.

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