AMPKα1 Kinase Assay

U*Light*-Acetyl-CoA Carboxylase (Ser79) Peptide and Europium-anti-phospho-Acetyl-CoA Carboxylase (Ser79) Antibody

Two LANCE *Ultra* companion products – two convenient sizes!

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*

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.

VALIDATED FOR KINASE: AMPKα1

POTENTIAL SUBSTRATE FOR KINASES:

AMP-activated subfamily of protein kinases

Europium-anti-phospho-Acetyl-CoA Carboxylase (Ser79) Antibody:

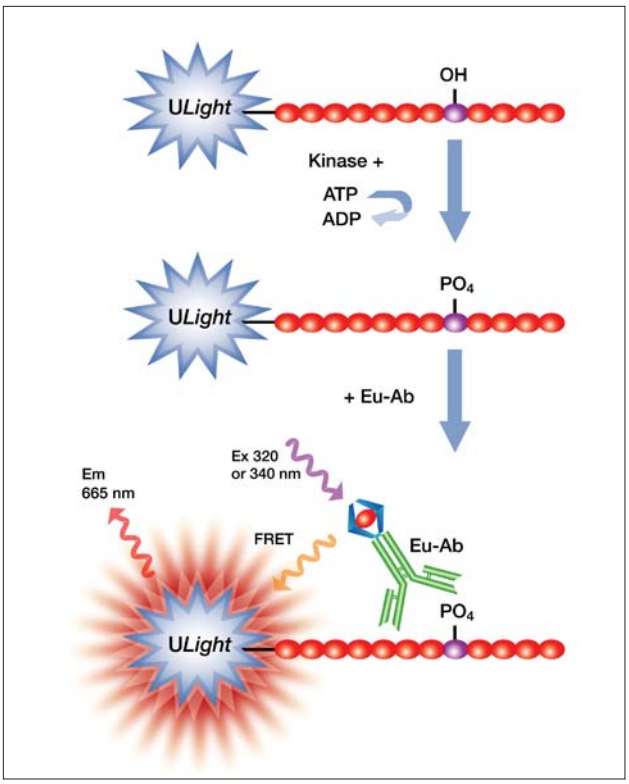
- TRF0208-D: 10 µg, 1,562* assay points
- TRF0208-M: 100 µg, 15,625* assay points

**40 fmol/assay point*

RECOGNIZED MOTIF:

SSM**pS**GL

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



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*, an innovative small molecular weight acceptor dye with a red-shifted fluorescent emission. In kinase assays, the binding of a 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.

Development of an AMPKα1 Kinase Assay

Additional reagents

AMPKα1 active	Carna # 02-113
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.

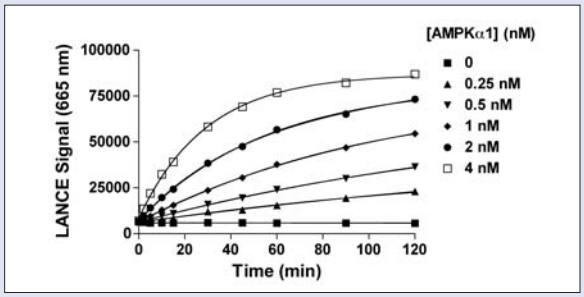
PerkinElmer® precisely.

Suggested procedure

- Dilute 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 and incubate at room temperature (RT).
- Stop kinase reactions by adding 5 µL of 40 mM EDTA prepared in 1X 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 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 and emission at 665 nm).

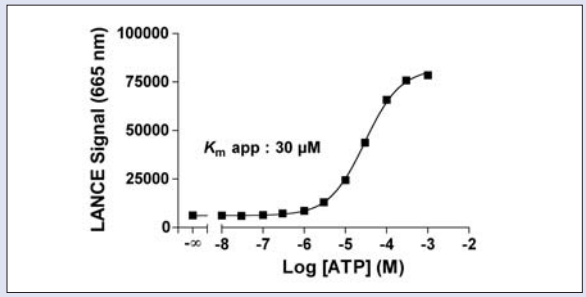
NOTE: Eu-labeled antibodies and EDTA can be premixed before use as a 2X concentrated Stop Solution/Detection Mix to minimize the number of liquid handling steps.

Experiment 1: Enzymatic Time Course



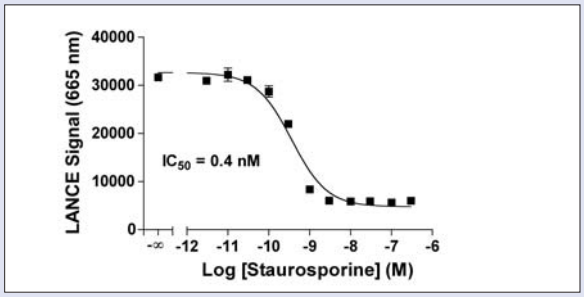
AMPKα1 enzyme was incubated at concentrations ranging from 0.25 to 4 nM with 50 nM U*Light*-Acetyl-CoA Carboxylase peptide and 20 µM ATP. Kinase reactions were terminated after 0 to 120 min by the addition of EDTA.

Experiment 2: ATP Titration



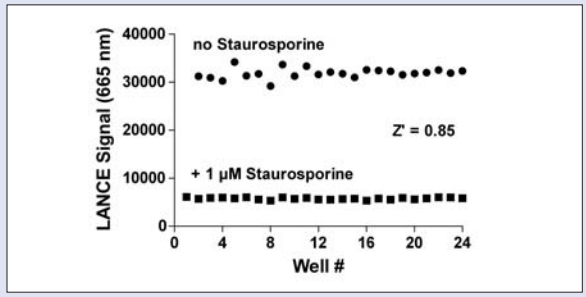
Serial dilutions of ATP ranging from 10 nM to 1 mM were added to 2 nM AMPKα1 and 50 nM U*Light*-Acetyl-CoA Carboxylase peptide. Kinase reactions were terminated after 30 min by the addition of EDTA.

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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