

U-TRF #12

LANCE *Ultra* AMPK α 1 Kinase Assay

LANCE[®] *Ultra* TR-FRET Technology

PerkinElmer, Inc.
Montreal, QC
Canada H3J 1R4

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 μ g, 1,562* assay points
- TRF0208-M: 100 μ g, 15,625* assay points

*40 fmol/assay point

ULight[™]-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:

SSMpSGL

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

Peptide Sequence:

CHMRSAMSGLHLVKRR

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 ULight[™], a small molecular weight acceptor dye with a red-shifted fluorescent emission.

In this technical note, we present the optimization of a AMPK α 1 kinase assay using a ULight-labeled peptide substrate. The binding of a Eu-labeled antibody directed against Ser79 phosphorylation of the SAMS peptide substrate brings the Eu donor and ULight acceptor dye molecules into close proximity. Upon irradiation at 320 or 340 nm, the energy from the Eu donor is transferred to the ULight acceptor dye which, in turn, generates light at 665 nm. The intensity of the light emission is proportional to the level of ULight substrate phosphorylation.

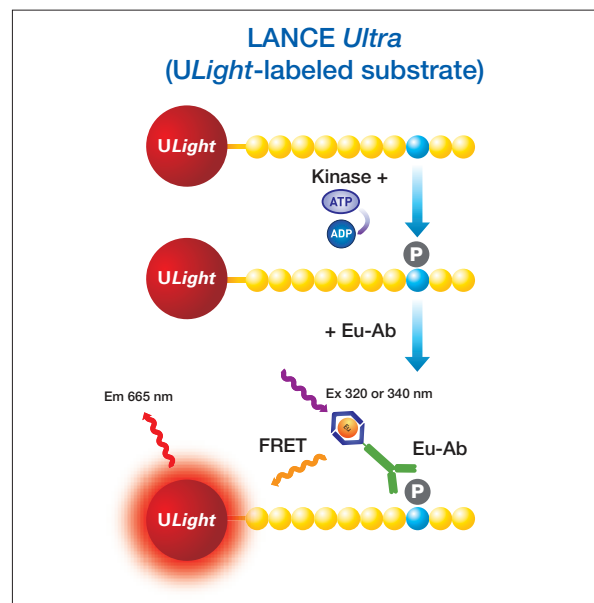


Figure 1. Schematic representation of the LANCE *Ultra* detection of a phosphorylated peptide substrate.

Development of an AMPK α 1 Kinase Assay

Reagents needed for this assay:

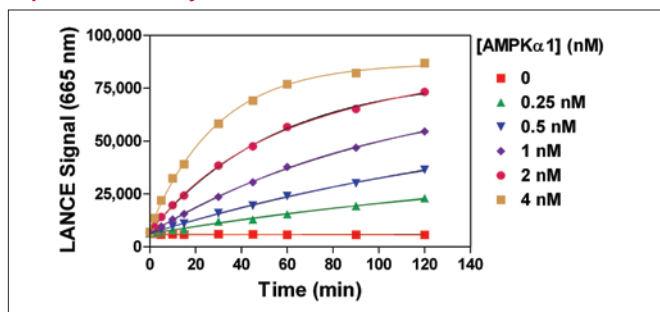
Europium-labeled anti-phospho-Acetyl-CoA Carboxylase (Ser79) Antibody	PerkinElmer # TRF0208
ULight-Acetyl-CoA Carboxylase (Ser79) Peptide	PerkinElmer # TRF0118
AMPK α 1, active	Carna # 02-113
LANCE [®] Detection Buffer, 10X	PerkinElmer # CR97-100
OptiPlate™-384, white	PerkinElmer # 6007299
TopSeal™-A film	PerkinElmer # 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 AMPK α 1 kinase, ATP, inhibitors and ULight-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 μ L of AMPK α 1 enzyme
 - 2.5 μ L of inhibitor or Kinase Buffer
 - 2.5 μ L of ULight-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 μ L of 40 mM EDTA prepared in 1X LANCE Detection Buffer (Stop Solution). Leave for 5 min at RT.
- Add 5 μ L 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).

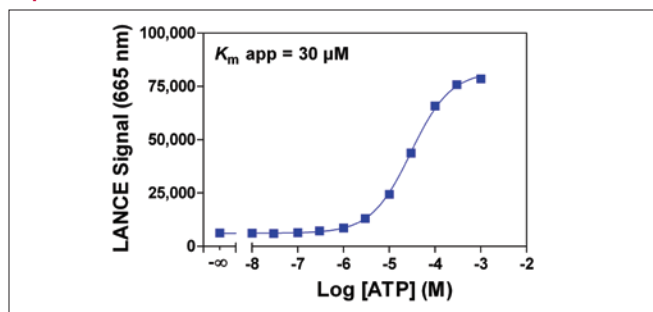
NOTE: Eu-labeled antibodies and EDTA can be premixed just before use as a 2X concentrated Stop Solution/Detection Mix to minimize the number of liquid handling steps.

Experiment 1: Enzymatic Titration and Time Course



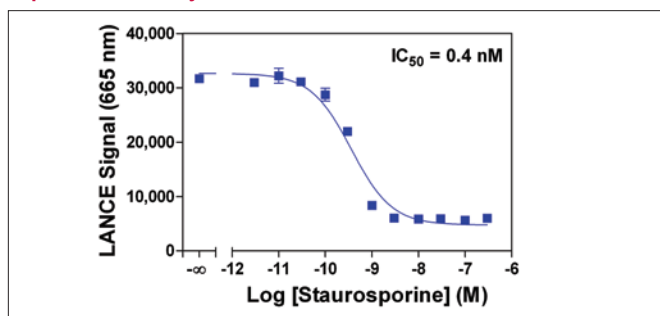
Enzymatic progress curves were produced by incubating AMPK α 1 enzyme at concentrations ranging from 0.25 to 4 nM with 50 nM ULight-Acetyl-CoA Carboxylase peptide and 20 μ M ATP. Kinase reactions were terminated at the indicated times by the addition of EDTA. Detection mix was added and signal read after 60 minutes.

Experiment 2: ATP Titration



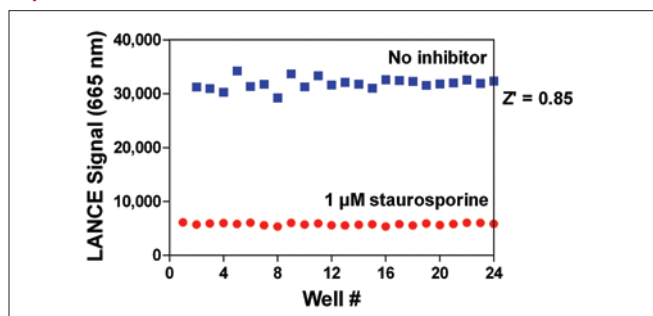
Serial dilutions of ATP ranging from 10 nM to 1 mM were added to 2 nM AMPK α 1 and 50 nM ULight-Acetyl-CoA Carboxylase peptide. Kinase reactions were terminated after 30 min by the addition of EDTA.

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 AMPK α 1, 50 nM ULight-Acetyl-CoA Carboxylase peptide and 30 μ M ATP. Kinase reactions were terminated after 30 min by the addition of EDTA.

Experiment 4: Z'-factor Determination



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

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com



For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

Copyright © 2007-2012, PerkinElmer, Inc. All rights reserved. PerkinElmer[®] is a registered trademark of PerkinElmer, Inc. All other trademarks are the property of their respective owners.