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CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

LANCE® *Ultra*

ULight™- labeled Acetyl-CoA carboxylase (Ser79) Peptide

Product number: TRF0133-M **Lot Number:** 3269563

Product Format:

TRF0133-M: 5 nmols (Lyophilized, add 1mL of water)

Manufacturing date: 4/29/2024 **Document version:** 1

Product Information

Phosphorylation Motif: RSAM**S**GLHL

Synthetic peptide derived from residues 73-85 of rat Acetyl CoA Carboxylase (also known as SAMS peptide) in which Ser77 is mutated to Ala; phosphorylation site: Ser79

Storage Buffer: 50 mM Tris-HCl (pH 7.4), 0.9% NaCl, 0.1% BSA and 0.05% sodium azide as preservative

Molecular Weight: 2 674

Stability: This product is stable for at least **9 months** from the manufacturing date when stored in its original packaging and the recommended storage conditions.

Storage Conditions: Store at -20°C. Store protected from light. Repeated freezing and thawing should be avoided.

Quality Control

The QC release specifications are based on spectrophotometric analysis of the labeled protein. We certify that results meet our quality release criteria.

Labeling Ratio: 1 (dye molecule/peptide)
Concentration: 13.4 µg/mL (5 µM)

Recommended Assay Conditions

AMPKα1 kinase: ATP titration
SUGGESTED METHOD:
(Specific applications might require optimization)

Reagent Preparation

- Prepare 1X Kinase Assay Buffer: 50 mM HEPES pH 7.5, 1 mM EGTA, 10 mM $MgCl_2$, 2 mM DTT and 0.01% Tween-20.
- Prepare a 2X AMPK1 solution: dilute enzyme to a concentration of 4 nM in Kinase Assay Buffer. Keep on ice.
- Prepare a 4X U*Light*-Acetyl CoA Carboxylase (Ser79) peptide solution: dilute U*Light*-Acetyl CoA Carboxylase (Ser79) to a concentration of 200 nM in Kinase Assay Buffer.
- Prepare a 4X ATP solution: dilute ATP to concentrations ranging from 40 nM to 4 mM (serial half-log dilutions) in Kinase Assay Buffer. Keep on ice.
- Prepare 1X Detection Buffer: dilute 0.5 mL of 10X Detection Buffer with 4.5 mL of H_2O.
- Prepare a 4X Stop solution*: dilute EDTA to a concentration of 40 mM in 1X Detection Buffer.
- Prepare a 4X Detection Mix: dilute Europium-anti-phospho-Acetyl CoA Carboxylase (Ser79) antibody to a concentration of 8 nM in 1X Detection Buffer.

Protocol

- Pipet 5 µL of 2X AMPK1 solution into a 384-well white OptiPlate-384 (2 nM final concentration).
- Add 2.5 µL of 4X U*Light*- Acetyl CoA Carboxylase (Ser79) solution (50 nM final concentration).
- Add 2.5 µL of 4X ATP solution (10 nM to 1 mM final concentrations).
- Cover plate with TopSeal-A and incubate 30 min at 23°C.
- Add 5 µL of 4X Stop solution* and incubate 5 min at 23°C.
- Add 5 µL of 4X Detection Mix (2 nM Eu-anti-phospho-Acetyl CoA Carboxylase (Ser79) final conc.).
- Cover plate with TopSeal-A and incubate 60 min at 23°C.
- Remove TopSeal-A and read in TR-FRET mode at 665 nm (excitation at 320 or 340 nm).

**Alternatively, the Stop solution and Detection Mix can be premixed and added together to the kinase reaction.*

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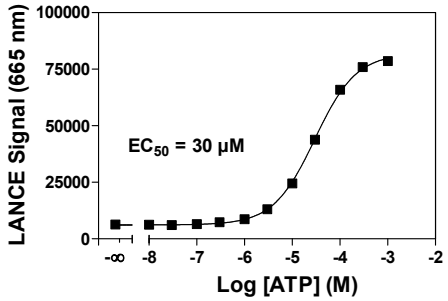
Typical Product Data

AMPKα1 kinase assay using U*Light*-Acetyl CoA Carboxylase (Ser79) Peptide and Eu-anti-phospho-Acetyl CoA Carboxylase (Ser79) Antibody obtained using the EnVision® Multilabel Reader:



Suggested Materials

	Supplier
• Substrate: U*Light*™- Acetyl CoA Carboxylase (Ser79)Pept.	Revvity Inc
• Antibody: Eu- anti- phospho-Acetyl CoA carboxylase(Ser79)	Revvity Inc
• Kinase: AMPKα1	Carna Biosciences
• Detection Buffer: LANCE® Detection Buffer, 10X	Revvity Inc
• Plate: OptiPlate™-384, white	Revvity Inc
• TopSeal™-A	Revvity Inc

TRF0133-R Rev01

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