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CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

LANCE® *Ultra*

ULight™-labeled CREBtide

Product number: TRF0107-M **Lot Number:** 3243662

Product Format: TRF0107-D: 0.5 nmole (100µL)

TRF0107-M: 5 nmoles (1mL)

Manufacturing date: 01/09/2024 **Document version:** 1

Product Information

Phosphorylation Motif: RRP**S**YRK

Synthetic peptide derived from human cAMP Response Element Binding (CREB) Protein. Phosphorylation site: Ser133

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

Molecular Weight: 2 740

Stability: This product is stable for at least **12 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: 1.0 (dye molecule/peptide)
Concentration: 13.7 µg/mL (5 µM)

Recommended Assay Conditions

MSK1: ATP titration

SUGGESTED METHOD:
(Specific applications might require optimization)

Reagent Preparation

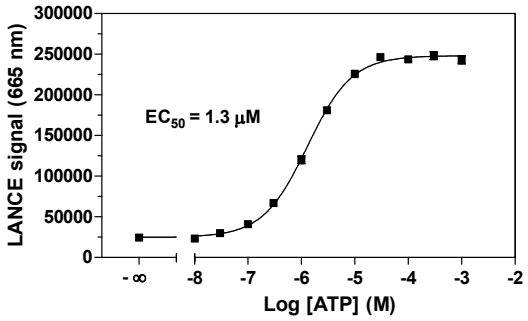
- Prepare 1X Kinase Assay Buffer: 50 mM Hepes pH 7.5, 1 mM EGTA, 10 mM $MgCl_2$, 2 mM DTT and 0.01% Tween-20.
- Prepare a 2X PKA solution: dilute the enzyme to a concentration of 20 pM in Kinase Assay Buffer. Keep on ice.
- Prepare a 4X mix of U*Light*-CREBtide: dilute U*Light*-CREBtide to a concentration of 200 nM in Kinase Assay Buffer.
- Prepare a 4X mix of ATP: 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: prepare a 40 mM EDTA solution in 1X Detection Buffer.
- Prepare a 4X Detection Mix: dilute the Eu-anti-phospho-CREB antibody to a concentration of 8 nM in 1X Detection Buffer.

Protocol

- Pipet 5 µL of 2X PKA solution into a 384-well white OptiPlate™-384 (10 pM final concentration).
- Add 2.5 µL of 4X U*Light*-CREBtide (50 nM final concentration).
- Add 2.5 µL of 4X ATP mix (10 nM to 1 mM final concentrations).
- Cover plate with TopSeal-A and incubate 60 min at 23°C.
- Add 5 µL of 4X Stop Solution and incubate 5 min at 23°C.
- Add 5 µL of Detection Mix (2 nM Eu-anti-phospho-CREB antibody final concentration) and mix.
- 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).

Typical Product Data

PKA kinase assay using U*Light*-CREBtide and Eu-anti-phospho-CREB antibody obtained using the EnVision® Multilabel Reader:



Suggested Materials

	Supplier
• Substrate: U*Light*™- CREBtide peptide	Revvity Inc
• Antibody: Eu- anti-phospho- CREB (Ser133)	Revvity Inc
• Kinase: PKA, catalytic subunit, recombinant	Upstate
• Detection Buffer: LANCE® Detection Buffer, 10X	Revvity Inc
• Plate: OptiPlate™-384, white	Revvity Inc
• TopSeal™-A	Revvity Inc

TRF0107-R Rev01

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