CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

LANCE® *Ultra*

Eu-W1024 labeled Anti-phospho-CREB (Ser133) Antibody

Product number:	TRF0200-C	**Lot Number:**	3303443
Product Format:	TRF0200-C: 1.7 µg		
Manufacturing date:	March 6, 2024	**Document version:**	1

Product Information

Antibody:	Europium-labeled rabbit monoclonal antibody recognizing phospho-Ser133 of the cAMP responsive element binding (CREB) protein.
Storage Buffer:	50 mM Tris-HCl (pH 7.4), 0.9% NaCl, 0.1% BSA and 0.05% sodium azide as preservative.
Molecular Weight:	160 000
Stability:	This product is stable for at least 24 months from the manufacturing date when stored in its original packaging and at the recommended storage conditions.
Storage Conditions:	Store at 4°C

Quality Control

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

Labeling Ratio: 6.28
Concentration: 100 µg/mL

Recommended Assay Conditions

PROTEIN KINASE A: ATP TITRATION

SUGGESTED METHOD:
(Specific applications might require optimization)

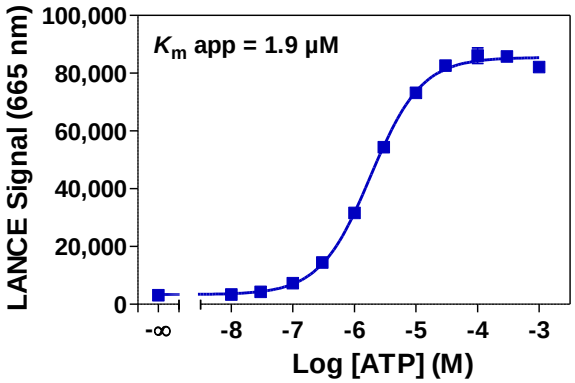
Reagent Preparation

- 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 5 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 24 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 (2.5 pM final concentration).
- Add 2.5 µL of 4X U*Light*- CREBtide (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 45 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-CREB antibody final conc.) 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



Suggested Materials

	Supplier
• Substrate: U*Light*™- CREBtide) Peptide	Revvity Inc
• Antibody: Eu- phospho-CREB (Ser133) Antibody	Revvity Inc
• Kinase: PKA, catalytic subunit, recombinant	Millipore
• Detection Buffer: LANCE® Detection Buffer, 10X	Revvity Inc
• Plate: OptiPlate™-384, white	Revvity Inc
• TopSeal™-A	Revvity Inc
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TRF0200-C-R Rev01

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