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CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

LANCE® *Ultra*

ULight™-labeled PLK (Ser 137) peptide

Product number: TRF0110-M **Lot Number:** 3215308

Product Format: TRF0110-D: 0.5 nmole (100µL)

TRF0110-M: 5 nmoles (1mL)

Manufacturing date: 11/01/2023 **Document version:** 1

Product Information

Phosphorylation Motif: RRR**S**LLE

Synthetic peptide containing the residues surrounding Ser137 of PLK; phosphorylation site: Ser137

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

Molecular Weight: 2 459

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: 12.3 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 Aurora A solution: dilute enzyme to a concentration of 8 nM in Kinase Assay Buffer. Keep on ice.
- Prepare a 4X U*Light*-PLK solution: dilute U*Light*-PLK 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-PLK to a concentration of 8 nM in 1X Detection Buffer.

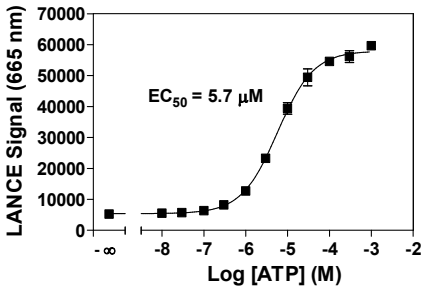
Protocol

- Pipet 5 µL of 2X Aurora A solution into a 384-well white OptiPlate-384 (4 nM final concentration).
- Add 2.5 µL of 4X U*Light*-PLK 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 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-PLK antibody final concentration).
- 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.*

Typical Product Data

Aurora A kinase assay using the U*Light*™-PLK Peptide and Eu-anti-phospho-PLK Antibody obtained using the EnVision® Multilabel Reader:



Suggested Materials

	Supplier
• Substrate: U*Light*™- PLK (Ser137) Peptide	Revvity Inc
• Antibody: Eu- anti-phospho-PLK (Ser137)	Revvity Inc
• Kinase: Aurora A, active	Upstate
• Detection Buffer: LANCE® Detection Buffer, 10X	Revvity Inc
• Plate: OptiPlate™-384, white	Revvity Inc
• TopSeal™-A	Revvity Inc

TRF0110-R Rev01

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