CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

LANCE® *Ultra*

Eu-W1024 labeled Anti-Phospho-DNA Topoisomerase 2-alpha (Thr1342) Antibody

Product number:	TRF0218-D	**Lot Number:**	3335649

Product Format: TRF0218-D: 10 µg

TRF0218-M: 100 µg

Manufacturing date:	2/27/2024	**Document version:**	1

Product Information

Antibody:	Europium-labeled mouse monoclonal antibody recognizing human DNA Topoisomerase 2-alpha phosphorylated at Thr1342.
Storage Buffer:	50 mM Tris-HCl (pH 7.4), 0.9% NaCl with 0.05% sodium azide as preservative and 0.1 % BSA
Molecular Weight:	160 000
Stability:	This product is stable for at least 18 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:	8.7 / 1
Concentration:	100 µg/mL (0.625 µM)

Recommended Assay Conditions

PLK3 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, 0.01% Tween-20 and 0.01% BSA.
- Prepare a 4X U*Light*-DNA Topoisomerase 2-alpha (Thr1342) Peptide solution: dilute U*Light*-DNA Topoisomerase 2-alpha (Thr1342) to a concentration of 200 nM in Kinase Assay Buffer.
- Prepare a 2X PLK3 solution: dilute enzyme to a concentration of 25 pM in Kinase Assay Buffer. Keep on ice.
- 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 24 mM in 1X Detection Buffer.
- Prepare a 4X Detection Mix: dilute Europium-anti-phospho-DNA Topoisomerase 2-alpha (Thr1342) Antibody to a concentration of 8 nM in 1X Detection Buffer.

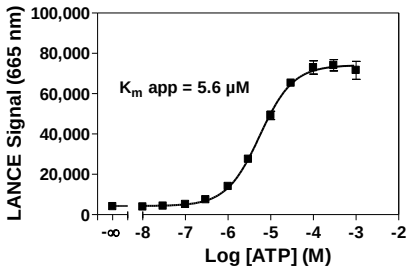
Protocol

- Pipet 5 µL of 2X PLK3 solution (12.5 pM final concentration).
- Add 2.5 µL of 4X U*Light*-DNA Topoisomerase 2-alpha (Thr1342) Peptide solution into a 384-well white OptiPlate-384 (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-DNA Topoisomerase 2-alpha (Thr1342) 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

PLK3 kinase assay using U*Light*™-Topo IIα (Thr1342) Peptide and Eu-anti-phospho-Topo IIα (Thr1342) Antibody



Suggested Materials

	Supplier
• Substrate: U*Light*™- Topo II (Thr1342) Peptide	Revvity Inc
• Antibody: Eu- anti-P-Topo II (Thr1342)	Revvity Inc
• Kinase: PLK3	Carna Biosciences
• Detection Buffer: LANCE® Detection Buffer, 10X	Revvity Inc
• Plate: OptiPlate™-384, white	Revvity Inc
• TopSeal™-A	Revvity Inc

TRF0218-R Rev01

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