

LANCE® Ultra

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

Product number: TRF0218-D Lot Number: 3318208

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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<sub>2</sub>, 2 mM DTT, 0.01% Tween-20 and 0.01% BSA.
- Prepare a 4X *ULight*-DNA Topoisomerase 2-alpha (Thr1342) Peptide solution: dilute *ULight*-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<sub>2</sub>O.
- 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.

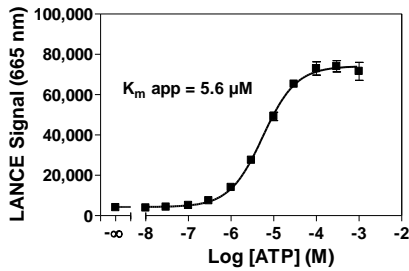
### Protocol

- Pipet 5 µL of 2X PLK3 solution (12.5 pM final concentration).
- Add 2.5 µL of 4X *ULight*-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 *ULight*<sup>TM</sup>-Topo II $\alpha$  (Thr1342) Peptide and Eu-anti-phospho-Topo II $\alpha$  (Thr1342) Antibody



### Suggested Materials

- Substrate: *ULight*<sup>TM</sup>- Topo II $\alpha$  (Thr1342) Peptide
- Antibody: Eu- anti-P-Topo II $\alpha$  (Thr1342)
- Kinase: PLK3
- Detection Buffer: LANCE<sup>®</sup> Detection Buffer, 10X
- Plate: OptiPlate<sup>TM</sup>-384, white
- TopSeal<sup>TM</sup>-A

### Supplier

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TRF0218-R Rev01

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