

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 MgCl₂, 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 H₂O.
- 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.

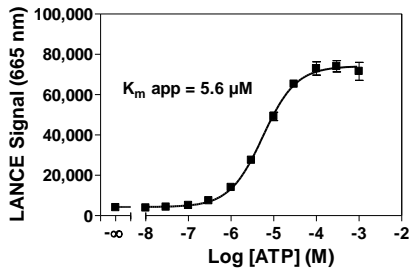
Protocol

- 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*TM-Topo II α (Thr1342) Peptide and Eu-anti-phospho-Topo II α (Thr1342) Antibody



Suggested Materials

- Substrate: *ULight*TM- Topo II α (Thr1342) Peptide
- Antibody: Eu- anti-P-Topo II α (Thr1342)
- Kinase: PLK3
- Detection Buffer: LANCE[®] Detection Buffer, 10X
- Plate: OptiPlateTM-384, white
- TopSealTM-A

Supplier

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TRF0218-R Rev01

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