

LANCE® Ultra

ULight™ - DNA Topoisomerase 2-alpha (Thr1342) Peptide**Product number:** TRF0130-M **Lot Number:** 3278020**Product Format:** TRF0130-D: 0.5 nmole

TRF0130-M: 5 nmoles

Manufacturing date: 03/27/2024 **Document version:** 1**Product Information****Phosphorylation Motif:** DEKTDDE

Synthetic peptide containing residues surrounding Thr1342 of human DNA Topoisomerase 2-alpha; phosphorylation site: 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:** 2 910**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:** 14.6 µg/mL (5 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.

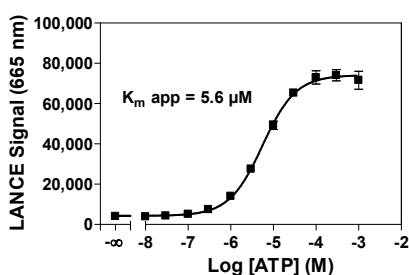
Protocol

- Pipet 5 μ L of 2X PLK3 solution (12.5 pM final concentration).
- Add 2.5 μ L of 4X *ULight*-DNA Topoisomerase 2-alpha (Thr1342) Peptide solution into a 384-well white OptiPlate-384 (50 nM final concentration).
- Add 2.5 μ L 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 μ L of 4X Stop solution* and incubate 5 min at 23°C.
- Add 5 μ L 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: <i>ULight</i> TM - Topo II α (Thr1342) Peptide | Supplier |
| • Antibody: Eu-anti- anti-P-Topo II α (Thr1342) | Revvity Inc |
| • Kinase: PLK3 | Revvity Inc |
| • Detection Buffer: LANCE [®] Detection Buffer, 10X | Carna Biosciences |
| • Plate: OptiPlate TM -384, white | Revvity Inc |
| • TopSeal TM -A | Revvity Inc |

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TRF0130-R Rev01

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