

LANCE® Ultra

ULight™ -labeled Histone H3 (Thr3/Ser10) Peptide**Product number:** TRF0125-M **Lot Number:** 3259857**Product Format:** TRF0125-D: 0.5 nmole (100µL)

TRF0125-M: 5 nmoles (1mL)

Manufacturing date: 02/14/2024 **Document version:** 1**Product Information****Phosphorylation Motif:** ARTKQTARK\$TGGK

Synthetic peptide containing the residues surrounding Thr3 and Ser10 of human Histone H3; phosphorylation sites: Thr3 and Ser10.

Storage Buffer: 50 mM Tris-HCl (pH 7.4), 0.9% NaCl, 0.1% BSA and 0.05% sodium azide as preservative**Molecular Weight:** 3 264**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 (dye molecule/peptide)**Concentration:** 16.3 µg/mL (5 µM)**Recommended Assay Conditions****RSK2 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 and 0.01% Tween-20.
- Prepare a 2X RSK2 solution: dilute enzyme to a concentration of 8 nM in 1X Kinase Assay Buffer. Keep on ice.
- Prepare a 4X ULight-Histone H3 (Thr3/Ser10) Peptide solution: dilute ULight-Histone H3 (Thr3/Ser10) Peptide to a concentration of 200 nM in 1X Kinase Assay Buffer.
- Prepare a 4X ATP solution: dilute ATP to concentrations ranging from 40 nM to 4 mM (serial half-log dilutions) in 1X 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 40 mM in 1X Detection Buffer.
- Prepare a 4X Detection Mix: dilute Europium-anti-phospho-Histone H3 (Thr3) Antibody to a concentration of 8 nM in 1X Detection Buffer.

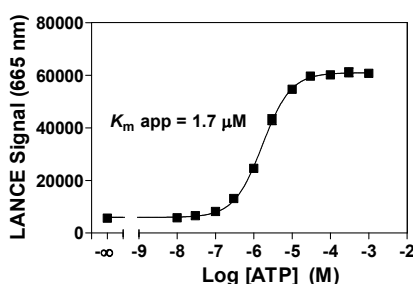
Protocol

- Pipet 5 μ L of 2X RSK2 solution into a 384-well white OptiPlate-384 (4 nM final concentration).
- Add 2.5 μ L of 4X *ULight*-Histone H3 (Thr3/Ser10) Peptide solution (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 45 min at 23°C.
- Add 5 μ L of 4X Stop solution* and incubate 5 min at 23°C.
- Add 5 μ L of 4X Detection Mix (2 nM Europium-anti-phospho-Histone H3 (Thr3) 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

RSK2 kinase assay using *ULight*-Histone H3 (Thr3/Ser10) Peptide and Eu-anti-phospho-Histone H3 (Thr3) Antibody obtained using the EnVision® Multilabel Reader:



Suggested Materials

	Supplier
• Substrate: <i>ULight</i> [™] - Histone H3 (Thr3/Ser10) Peptide	Revvity Inc
• Antibody: Eu-anti- phospho-Histone H3 (Thr3) Antibody	Revvity Inc
• Kinase: RSK2	Carna Biosciences
• Detection Buffer: LANCE [®] Detection Buffer, 10X	Revvity Inc
• Plate: OptiPlate [™] -384, white	Revvity Inc
• TopSeal [™] -A	Revvity Inc

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TRF0125-R Rev01

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Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000 www.revvity.com

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