TECHNICAL DATA SHEET

LANCE® *Ultra* Research Reagents

Caution: For Laboratory Use. A product for research purposes only

ULight™-labeled Histone H3 (Thr3/Ser10) Peptide

Product No.: TRF0125-D

Lot No.: 2913204

Material Provided

Format:

TRF0125-D	0.5 nmole (1 000 assay points*)
TRF0125-M	5 nmoles (10 000 assay points*)

*Assuming 0.5 pmol/ assay point

Volume: 100 µL (TRF0125-D) or 1 mL (TFR0125-M)

Manufacturing Date: **June 17, 2021**

Product Information

Phosphorylation Motif: AR**T**KQTARK**S**TGGK
Synthetic peptide containing the residues surrounding Thr3 and Ser10 of human Histone H3; phosphorylation sites: Thr3 and Ser10.

Molecular Weight: 3 264

Storage Buffer: 50 mM Tris-HCl (pH 7.4), 0.9% NaCl, 0.1% BSA and 0.05% sodium azide as preservative

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.

Safety Note: The storage buffer contains sodium azide (NaN_3) as a preservative. Disposal of all waste should be in accordance with local regulations.

Quality Control

The QC release specifications are based on spectrophotometric analysis of the labeled peptide. We certify that these results meet our quality release criteria.

Labeling Ratio: 1.0 (dye molecule/peptide)

Concentration: 16.3 µg/mL (5 µM)

PerkinElmer® For the Better

Recommended Assay Conditions

RSK2 kinase: ATP titration

Reagent Preparation:

- Prepare 1X Kinase Assay Buffer: 50 mM HEPES pH 7.5, 1 mM EGTA, 10 mM $MgCl_2$, 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 U*Light*-Histone H3 (Thr3/Ser10) Peptide solution: dilute U*Light*-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 H_2O.
- 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.

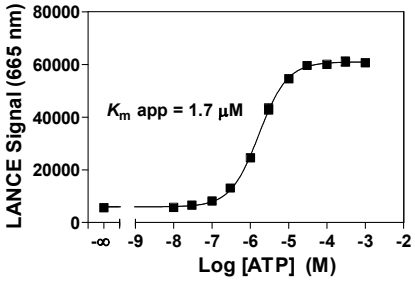
Protocol:

- Pipet 5 µL of 2X RSK2 solution into a 384-well white OptiPlate-384 (4 nM final concentration).
- Add 2.5 µL of 4X U*Light*-Histone H3 (Thr3/Ser10) Peptide solution (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 45 min at 23°C.
- Add 5 µL of 4X Stop solution* and incubate 5 min at 23°C.
- Add 5 µL 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 ATP Titration Data

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



Suggested Materials

	Supplier	Cat. No.
• Substrate: U*Light*™- Histone H3 (Thr3/Ser10) Peptide	PerkinElmer	TRF0125
• Antibody: Eu- anti-phospho-Histone H3 (Thr3) Antibody	PerkinElmer	TRF0211*
• Kinase: RSK2	Carna Biosciences	01-150
• Detection Buffer: LANCE® Detection Buffer, 10X	PerkinElmer	CR97-100
• Plate: OptiPlate™-384, white	PerkinElmer	6007299
• TopSeal™:TopSeal-A	PerkinElmer	6050195

**This peptide can also be used with Eu-anti-phospho-Histone H3 (Ser10) Antibody (PerkinElmer # TRF0210)*

Please visit our website for additional resource:

www.perkinelmer.com/LANCE

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®