CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

LANCE® *Ultra*

Eu-W1024 labeled Anti-phospho-Histone H3 (Thr3) Antibody

Product number:	TRF0211-D	**Lot Number:**	3312183

Product Format: TRF0211-D: 10 µg

TRF0211-M: 100 µg

Manufacturing date: 4/17/2024 **Document version:** 1

Product Information

Antibody:	Europium-labeled rabbit monoclonal antibody recognizing phospho-Thr3 in human Histone H3.
Storage Buffer:	50 mM Tris-HCl (pH 7.4), 0.9% NaCl, 0.1% BSA and 0.05% sodium azide as preservative
Molecular Weight:	160 000
Stability:	This product is stable for at least 12 months from the manufacturing date when stored in its original packaging and at the recommended storage conditions.
Storage Conditions:	Store at -20°C. Repeated freezing and thawing should be avoided.

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: 7.0 / 1
Concentration: 0.625 µM , 100 µg/mL

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 $MgCl_2$, 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 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.

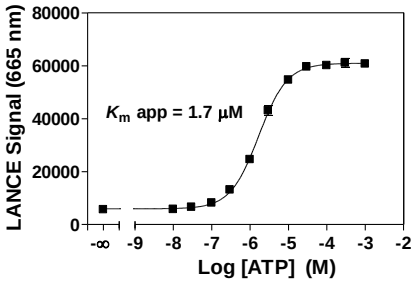
Protocol

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



Suggested Materials

	Supplier
• Substrate: U*Light*™- 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

TRF0211-R Rev01

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