

LANCE® Ultra

**Eu-W1024 labeled Anti-phospho-Histone H3 (Thr3) Antibody****Product number:** TRF0211-D/M **Lot Number:** 3249107 / 3240562**Product Format:** TRF0211-D: 10 µg

TRF0211-M: 100 µg

**Manufacturing date:** 1/24/2024**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:** 7.4**Concentration:** 100 µg/mL (0.625 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 MgCl<sub>2</sub>, 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 Kinase Assay Buffer. Keep on ice.
- Prepare 1X Detection Buffer: dilute 0.5 mL of 10X Detection Buffer with 4.5 mL of H<sub>2</sub>O.
- 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.

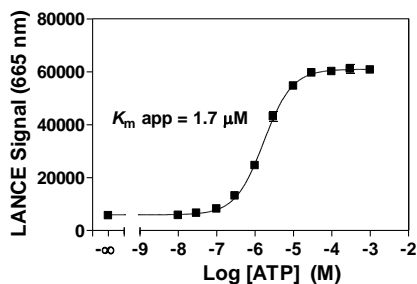
### Protocol

- Pipet 5 µL of 2X RSK2 solution into a 384-well white OptiPlate-384 (4 nM final concentration).
- Add 2.5 µL of 4X *ULight*-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 *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> <sup>™</sup> - Histone H3 (Thr3/Ser10) Peptide | Revvity Inc       |
| • Antibody: Eu- anti-phospho-Histone H3 (Thr3) Antibody                   | Revvity Inc       |
| • Kinase: RSK2  | Carna Biosciences |
| • Detection Buffer: LANCE <sup>®</sup> Detection Buffer, 10X              | Revvity Inc       |
| • Plate: OptiPlate <sup>™</sup> -384, white                               | Revvity Inc       |
| • TopSeal <sup>™</sup> -A   | Revvity Inc       |

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