

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

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

**Product No.:** TRF0211-D / TRF0211-M

**Lot No.:** 2519266

### **Material Provided**

**Format:** TRF0211-D 10 µg (1 562 assay points\*)  
TRF0211-M 100 µg (15 625 assay points\*)  
\*Assuming 40 fmol/ assay point

**Volume:** 100 µL (TRF0211-D) or 1 mL (TFR0211-M)

**Manufacturing Date:** **September 26, 2018**

### **Product Information**

**Antibody:** Europium-labeled rabbit monoclonal antibody recognizing phospho-Thr3 in human Histone H3.

**Molecular Weight:** 160 000

**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. Repeated freezing and thawing should be avoided.

**Safety Note:** The storage buffer contains sodium azide (NaN<sub>3</sub>) 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 antibody. We certify that these results meet our quality release criteria.

**Labeling Ratio:** 6.69 (Eu/Ab)

**Concentration:** 100 µg/mL (0.625 µM)

## 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<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 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<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.

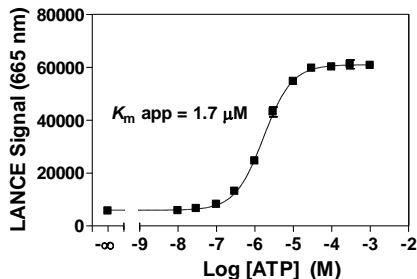
#### Protocol:

- 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 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 *ULight*-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: <i>ULight</i> ™- 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

Please visit our website for additional resource:

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