

LANCE® Ultra

**ULight™ -labeled p70S6K (Thr389) Peptide**

Product number: TRF0126-M Lot Number: 3208814

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Product Format: TRF0126-D: 0.5 nmole (100µL)

TRF0126-M: 5 nmoles (1mL)

Manufacturing date: 11/06/2023 Document version: 1

**Product Information**

**Phosphorylation Motif:** LGFTYVAP  
Synthetic peptide containing residues surrounding Thr389 of human p70 S6K; phosphorylation site: Thr389.

**Storage Buffer:** 50 mM Tris-HCl, pH 7.4, 0.9% NaCl, 0.1% BSA, 20% Acetonitrile and 0.05% sodium azide as preservative

**Molecular Weight:** 3 351

**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 -80°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 antibody. We certify that results meet our quality release criteria.

**Labeling Ratio:** 1.0 (dye molecule/peptide)

**Concentration:** 16.8 µg/mL (5 µM)

**Recommended Assay Conditions**

## COT (MAP3K8) Kinase Assay

### 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>, 3 mM MnCl<sub>2</sub>, 2 mM DTT and 0.01% Tween-20. **Note:** MnCl<sub>2</sub> might not be required for other kinases.
- Prepare a 2X COT solution: dilute the enzyme to a concentration of 20 nM in Kinase Assay Buffer. Keep on ice.
- Prepare a 4X ULight-p70 S6K (Thr389) Peptide solution: dilute ULight-p70 S6K (Thr389) Peptide to a concentration of 200 nM in 1X Kinase Assay Buffer.
- Prepare three separate 4X ATP solutions: dilute ATP to concentrations of 0, 40 or 400 μM in 1X Kinase Assay Buffer. Keep on ice.
- Prepare 1X Detection Buffer: dilute 0.5 mL of 10X Detection Buffer in 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 the Eu-anti-phospho-p70 S6K (Thr389) Antibody to a concentration of 8 nM in 1X Detection Buffer.

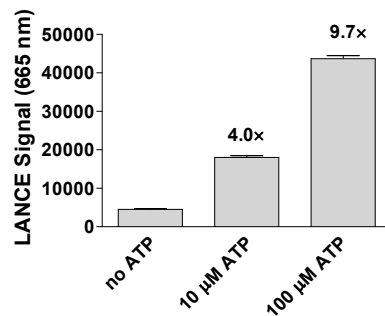
### Protocol

- Pipet 5 μL of 2X COT solution into a 384-well white OptiPlate™-384 (10 nM final concentration).
- Add 2.5 μL of the 4X ULight-p70 S6K (Thr389) Peptide (50 nM final concentration).
- Add 2.5 μL of 4X ATP solution (0, 10 and 100 μM final concentrations).
- Cover plate with TopSeal-A and incubate 90 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 Eu-anti-phospho-p70 S6K (Thr389) 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

COT Kinase Assay using ULight-p70 S6K (Thr389) Peptide and Eu-anti-phospho-p70 S6K (Thr389) Antibody obtained using the EnVision® Multilabel Reader:



## Suggested Materials

	Supplier
• Substrate: p70 S6K (Thr389) Peptide	Revvity Inc
• Antibody: Eu- anti-phospho-p70 S6K (Thr389) Antibody	Revvity Inc
• Kinase: COT (MAP3K8)	Carna Biosciences
• Detection Buffer: LANCE <sup>®</sup> Detection Buffer, 10X	Revvity Inc
• Plate: OptiPlate™-384, white	Revvity Inc
• TopSeal™-A	Revvity Inc

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TRF0126-R Rev01

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