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CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

LANCE® *Ultra*

ULight™-labeled p70S6K (Thr389) Peptide

Product number: TRF0126-D **Lot Number:** 3285852

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

TRF0126-M: 5 nmoles (1mL)

Manufacturing date: 06/18/2024 **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 $MgCl_2$, 3 mM $MnCl_2$, 2 mM DTT and 0.01% Tween-20. **Note:** $MnCl_2$ 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 U*Ligh*t-p70 S6K (Thr389) Peptide solution: dilute U*Ligh*t-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_2O.
- 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.

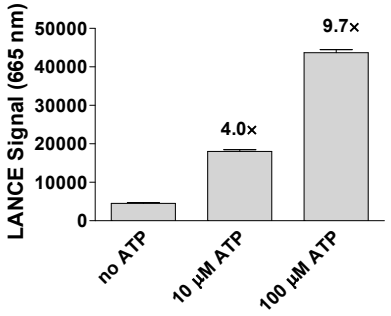
Protocol

- 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 U*Light*-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 U*Light*-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® Detection Buffer, 10X	Revvity Inc
• Plate: OptiPlate™-384, white	Revvity Inc
• TopSeal™-A	Revvity Inc

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