

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

**Eu-W1024-labeled Anti-Phospho-40S ribosomal protein S6
(Ser235/236) Antibody****Product No.: TRF0217-D / TRF0217-M****Lot No.: 3061864****Material Provided**

Format: TRF0217-D 10 µg (1 562 assay points*)
TRF0217-M 100 µg (15 625 assay points*)
*Assuming 40 fmol/assay point

Manufacturing Date: August 31, 2022

Product Information

Antibody: Europium-labeled rabbit monoclonal antibody recognizing human 40S ribosomal protein S6 phosphorylated at both Ser235 and Ser236.

Storage Buffer: 50 mM Tris-HCl (pH 7.4), 0.9% NaCl with 0.05% sodium azide as preservative and 0.1 % BSA

Molecular Weight: 160 000

Stability: This product is stable for at least **18 months** from the manufacturing date when stored in its original packaging and at the recommended storage conditions.

Storage Conditions: Store at 4°C

Safety Note: The storage buffer contains sodium azide (NaN₃) as a preservative. Disposal of all waste should be in accordance with local regulations.

Quality Control

This product passed QC release specifications based on spectrophotometric analysis of the labeled antibody.

Labeling Ratio: 6.8 (Eu/Ab)

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 MgCl₂, 2 mM DTT and 0.01% Tween-20.
- Prepare a 4X *ULight*-40S ribosomal protein S6 (pSer235/Ser236) Peptide solution: dilute *ULight*-40S ribosomal protein S6 (pSer235/236) to a concentration of 200 nM in Kinase Assay Buffer.
- Prepare a 2X RSK2 solution: dilute enzyme to a concentration of 50 pM in Kinase Assay Buffer. Keep on ice.
- 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 H₂O.
- Prepare a 4X Stop solution*: dilute EDTA to a concentration of 24 mM in 1X Detection Buffer.
- Prepare a 4X Detection Mix: dilute Europium-anti-phospho-40S ribosomal protein S6 (Ser235/Ser236) Antibody to a concentration of 8 nM in 1X Detection Buffer.

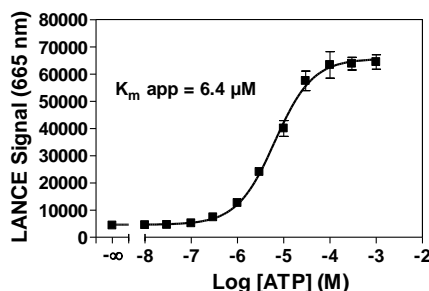
Protocol

- Pipet 5 µL of 4X RSK2 solution (25 pM final concentration).
- Add 2.5 µL of 2X *ULight*-40S ribosomal protein S6 (pSer235/Ser236) peptide solution into a 384-well white OptiPlate-384 (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 60 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 Eu-anti-phospho-40S ribosomal protein S6 (Ser235/236) 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 obtained using the EnVision® Multilabel Reader:

RSK2 kinase assay using *ULight*TM-rpS6 (pSer235/Ser236) Peptide and
Eu-anti-phospho-rpS6 (Ser235/236) Antibody



Suggested Materials

	Supplier	Cat. No.
• Substrate: <i>ULight</i> TM -rpS6 (pSer235/Ser236) Peptide	PerkinElmer	TRF0129
• Antibody: Europium-anti-P-rpS6 (Ser235/236)	PerkinElmer	TRF0217
• Kinase: RSK2 (RPS6KA3)	Carna Biosciences	01-150
• Detection Buffer: LANCE® Detection Buffer, 10X	PerkinElmer	CR97-100
• Plate: OptiPlate TM -384, white	PerkinElmer	6007299
• TopSeal TM -A	PerkinElmer	6050195

Please visit our website for additional resource:

www.perkinelmer.com/LANCE

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