TECHNICAL DATA SHEET

LANCE® *Ultra* Research Reagents

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

Lot No.: 3200127

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: $100 \mu g/mL (0.625 \mu M)$



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

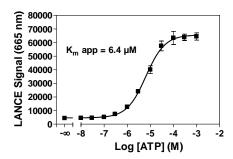
- Pipet 5 μL of 4X RSK2 solution (25 pM final concentration).
- Add 2.5 μL of 2X U*Light*-40S ribosomal protein S6 (pSer235/Ser236) peptide solution into a 384-well white OptiPlate-384 (50 nM final concentration).
- Add 2.5 µL of 4X ATP solution (10 nM to 1 mM final concentrations).
- Cover plate with 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[™]-rpS6 (pSer235/Ser236) Peptide and Eu-anti-phospho-rpS6 (Ser235/236) Antibody



Suggested Materials

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•	Substrate: U <i>Light</i> ™-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™-384, white	PerkinElmer	6007299
•	TopSeal™-A	PerkinElmer	6050195

Supplier

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