

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

Eu-W1024-labeled Anti-Phospho-eIF4E-binding protein 1 (Thr37/46) Antibody

Product No.: TRF0216-D / TRF0216-M

Lot No.: 2738348

Material Provided

Format: TRF0216-D 10 µg (1 562 assay points*)
TRF0216-M 100 µg (15 625 assay points*)
*Assuming 40 fmol/assay point

Manufacturing Date: **June 26, 2020**

Product Information

Antibody: Europium-labeled rabbit monoclonal antibody recognizing human eukaryotic translation initiation factor 4E-binding protein 1 phosphorylated at Thr37 or Thr46.

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: 7.58 (Eu/Ab)

Concentration: 100 µg/mL (0.625 µM)

Recommended Assay Conditions

mTOR 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, 3 mM MnCl₂, 2 mM DTT and 0.01% Tween-20.
- Prepare a 4X *ULight*-eIF4E-binding protein 1 (Thr37/46) Peptide solution: dilute *ULight*-eIF4E-binding protein 1 to a concentration of 200 nM in Kinase Assay Buffer.
- Prepare a 2X mTOR solution: dilute enzyme to a concentration of 1 nM 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 32 mM in 1X Detection Buffer.
- Prepare a 4X Detection Mix: dilute Europium-anti-phospho-eIF4E-binding protein 1 (Thr37/46) Antibody to a concentration of 8 nM in 1X Detection Buffer.

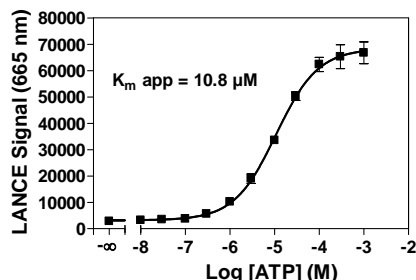
Protocol

- Pipet 5 µL of 2X mTOR solution (0.5 nM final concentration).
- Add 2.5 µL of 4X *ULight*-eIF4E-binding protein 1 (Thr37/46) 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-eIF4E-binding protein 1 (Thr37/46) 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:

mTOR kinase assay using *ULight*TM-4E-BP1 (Thr37/46) Peptide and Eu-anti-phospho-4E-BP1 (Thr37/46) Antibody



Suggested Materials

- Substrate: *ULight*TM-4E-BP1 (Thr37/46) Peptide
- Antibody: Europium-anti-P-4E-BP1 (Thr37/46)
- Kinase: mTOR, active
- Detection Buffer: LANCE[®] Detection Buffer, 10X
- Plate: OptiPlateTM-384, white
- TopSealTM-A

Supplier

Cat. No.

| | |
|-------------------|----------|
| PerkinElmer | TRF0128 |
| PerkinElmer | TRF0216 |
| Millipore/Upstate | 14-770 |
| PerkinElmer | CR97-100 |
| PerkinElmer | 6007299 |
| PerkinElmer | 6050195 |

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