

LANCE® Ultra

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

**Product number:** TRF0216-M **Lot Number:** 3272913

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**Product Format:** TRF0216-D: 10 µg  
TRF0216-M: 100 µg

**Manufacturing date:** March 12, 2024 **Document version:** 1

## 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.

## 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:** 6.23  
**Concentration:** 100 µg/mL (0.625µM)

## 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 MgCl<sub>2</sub>, 3 mM MnCl<sub>2</sub>, 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 H<sub>2</sub>O.
- 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.

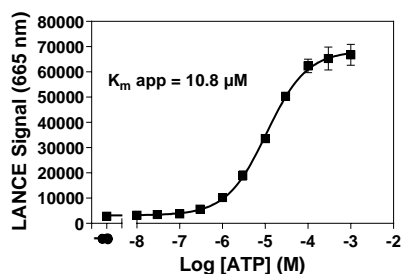
### Protocol

- Pipet 5  $\mu$ L of 2X mTOR solution (0.5 nM final concentration).
- Add 2.5  $\mu$ L 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  $\mu$ L 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  $\mu$ L of 4X Stop solution\* and incubate 5 min at 23°C.
- Add 5  $\mu$ L 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 Product Data

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



## Suggested Materials

- Substrate: *ULight*<sup>TM</sup>-4E-BP1 (Thr37/46) Peptide
- Antibody: Europium-anti-P-4E-BP1 (Thr37/46)

### Supplier

Revvity Inc  
Revvity Inc

- Kinase: mTOR, active
- Detection Buffer: LANCE® Detection Buffer, 10X
- Plate: OptiPlate™-384, white
- TopSeal™-A

Millipore/Upstate  
Revvity Inc  
Revvity Inc  
Revvity Inc

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TRF0216-R Rev01

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