

LANCE® Ultra

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

Product number: TRF0216-M Lot Number: 3296413

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

Manufacturing date: 6/26/2024 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: 7.6 / 1
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.

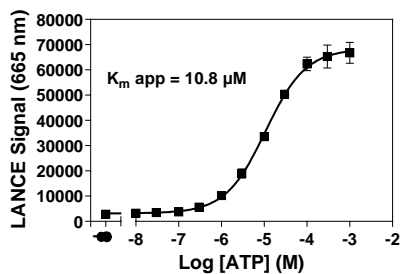
Protocol

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

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

The logo for Revvity, featuring the word "revvity" in a lowercase, sans-serif font.

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000 www.revvity.com

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