

LANCE® Ultra

ULight™ - eIF4E-binding protein 1 (Thr37/46) Peptide**Product number:** TRF0128-D **Lot Number:** 3254955**Product Format:** TRF0128-D: 0.5 nmole

TRF0128-M: 5 nmoles

Manufacturing date: 01/22/2024 **Document version:** 1**Product Information****Phosphorylation Motif:** STTPGGTLFSTTPG

Synthetic peptide containing residues surrounding Thr37 and Thr46 of human eukaryotic translation initiation factor 4E-binding protein 1; phosphorylation sites: Thr37 and 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:** 3 588**Stability:** This product is stable for at least **12 months** from the manufacturing date when stored in its original packaging and the recommended storage conditions.**Storage Conditions:** Store at -20°C. Store protected from light. Repeated freezing and thawing should be avoided.**Quality Control**

The QC release specifications are based on spectrophotometric analysis of the labeled protein. We certify that results meet our quality release criteria.

Labeling Ratio: 1.0 (dye molecule/peptide)**Concentration:** 17.9 µg/mL (5 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.

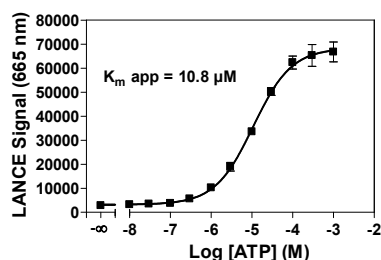
Protocol

- 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*[™]-4E-BP1 (Thr37/46) Peptide and Eu-anti-phospho-4E-BP1 (Thr37/46) Antibody



Suggested Materials

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

Supplier

Revvity Inc
Revvity Inc
Millipore/Upstate
Revvity Inc
Revvity Inc
Revvity Inc

The information provided in this document is valid for the specified lot number and date of analysis. This information is for reference purposes only and does not constitute a warranty or guarantee of the product's suitability for any specific use. Revvity, Inc., its subsidiaries, and/or affiliates (collectively, "Revvity") do not assume any liability for any errors or damages arising from the use of this document or the product described herein. REVVITY EXPRESSLY DISCLAIMS ALL WARRANTIES, INCLUDING WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, REGARDLESS OF WHETHER ORAL OR WRITTEN, EXPRESS OR IMPLIED, ALLEGEDLY ARISING FROM ANY USAGE OF ANY TRADE OR ANY COURSE OF DEALING, IN CONNECTION WITH THE USE OF INFORMATION CONTAINED HEREIN OR THE PRODUCT ITSELF.

TRF0128-R Rev01

revvity

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

(800) 762-4000 www.revvity.com

For a complete listing of our global offices, visit www.revvity.com
Copyright ©2023, Revvity, Inc. All rights reserved.