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

**Product No.:** TRF0216-D / TRF0216-M

**Lot No.:** 2639589

### **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:** **September 12, 2019**

### **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 (NaN<sub>3</sub>) 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:** 7.49

**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 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.

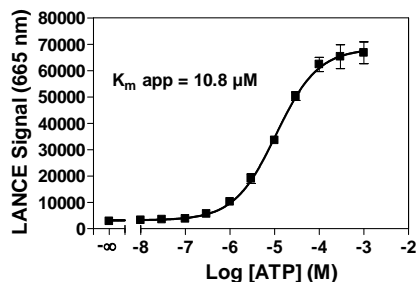
#### Protocol

- Pipet 5 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*<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)
- Kinase: mTOR, active
- Detection Buffer: LANCE<sup>®</sup> Detection Buffer, 10X
- Plate: OptiPlate<sup>TM</sup>-384, white
- TopSeal<sup>TM</sup>-A

### Supplier

### Cat. No.

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

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PerkinElmer, Inc.  
940 Winter Street  
Waltham, MA 02451 USA  
P: (800) 762-4000 or  
(+1) 203-925-4602  
[www.perkinelmer.com](http://www.perkinelmer.com)



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