TECH NOTE *U*-TRF #26

mTOR Kinase Assay

U*Light*-p70 S6K (Thr389) Peptide & Europium-anti-phospho-p70 S6K (Thr389) Antibody

Two LANCE *Ultra* companion products - two convenient sizes!

U*Light*™-p70 S6K (Thr389) Peptide:

- TRF0126-D: 0.5 nmole, 1,000 assay points*
- TRF0126-M: 5 nmoles, 10,000 assay points*

**0.5 pmol/assay point*

PEPTIDE MOTIF:
FLGFTYVAP
Synthetic peptide containing the residues surrounding Thr389 of human p70 S6K; phosphorylation site: Thr389.

VALIDATED FOR KINASE: CDK6/CycD3, COT, HGK, IKKα, IKKε, IRAK1, IRAK4, MAP4K2, MINK, MST1, NEK1, NEK2, NEK6, NEK7, PEK, PLK1, TAOK2

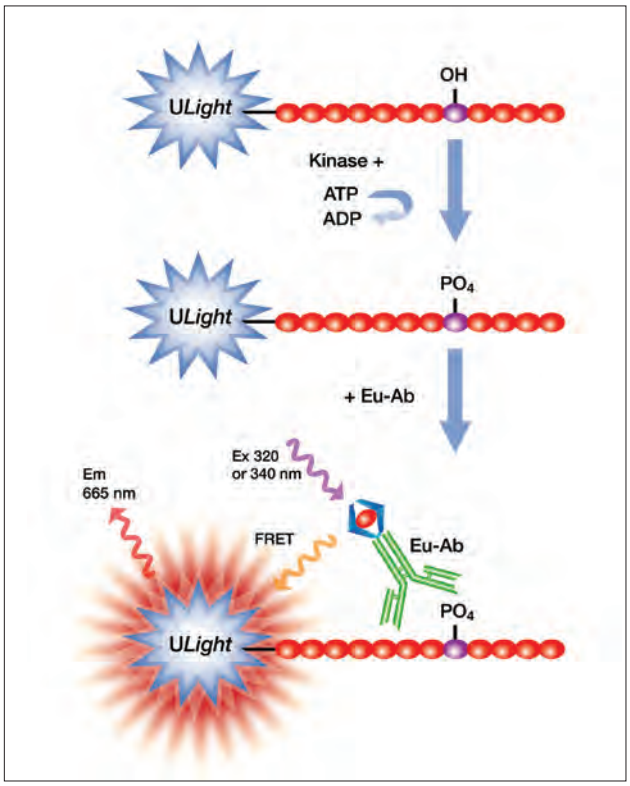
Europium-anti-phospho-p70 S6K (Thr389) Antibody:

- TRF0214-D: 10 µg, 1,562 assay points*
- TRF0214-M: 100 µg, 15,625 assay points*

**40 fmol/assay point*

RECOGNIZED MOTIF:
FLGFTYVAP

Europium-labeled mouse monoclonal antibody recognizing phospho-Thr389 in human p70 S6 K.



LANCE *Ultra* Kinase Assays

LANCE® *Ultra* time-resolved fluorescence resonance energy transfer (TR-FRET) assays use a proprietary europium chelate donor dye, W-1024 (Eu), with U*Light*, an innovative small molecular weight acceptor dye with a red-shifted fluorescent emission. In kinase assays, the binding of an Eu-labeled anti-phospho-substrate antibody to the phosphorylated U*Light*-labeled substrate brings donor and acceptor molecules into close proximity.

After irradiation of the kinase reaction at 320 or 340 nm, the energy from the Eu donor is transferred to the U*Light* acceptor dye which, in turn, generates light at 665 nm. The intensity of the light emission is proportional to the level of U*Light*-substrate phosphorylation.

Development of a mTOR Kinase Assay

Additional reagents

mTOR	Upstate # 14-770
LANCE Detection Buffer, 10X	PerkinElmer # CR97-100
OptiPlate™-384, white	PerkinElmer # 6007299
TopSeal™ -A	PerkinElmer # 6005185

Kinase Buffer: 50 mM HEPES pH 7.5, 1 mM EGTA, 3 mM $MnCl_2$, 10 mM $MgCl_2$, 2 mM DTT and 0.01% Tween-20.
Note: $MnCl_2$ might not be required for other kinases.

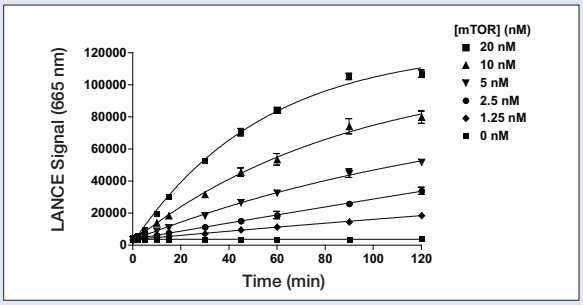
PerkinElmer®

Suggested procedure

- Dilute the mTOR enzyme, ATP, inhibitors and U*Light*-p70 S6K Peptide in Kinase Buffer.
- Prepare a 4X Detection Mix by diluting the Eu-anti-phospho-p70 S6K Antibody to 8 nM in 1X LANCE Detection Buffer.
- Add to the wells of a white Optiplate-384:
  - 5 µL of mTOR enzyme
  - 2.5 µL of inhibitor or Kinase Buffer
  - 2.5 µL of U*Light*-p70 S6K Peptide/ATP mix (for ATP titration, ATP dilutions are added separately in Kinase Buffer).
- Cover the plate with TopSeal-A and incubate at room temperature (RT).
- Stop kinase reactions by adding 5 µL of 40 mM EDTA prepared in 1X Detection Buffer (Stop Solution). Leave for 5 min at RT.
- Add 5 µL of Detection Mix (Eu-anti-phospho-p70 S6K Antibody at a final concentration of 2 nM).
- Cover with TopSeal-A and incubate for 1 h at RT.
- Remove TopSeal-A and read signal with the EnVision® Multilabel Reader in TR-FRET mode (excitation at 320 nm & emission at 665 nm).

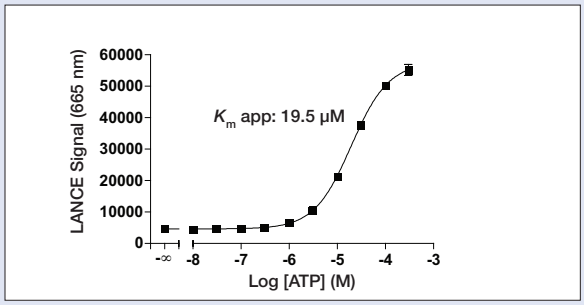
NOTE: Eu-labeled antibodies and EDTA can be premixed before use as a 2X concentrated Stop Solution/Detection mix to minimize the number of liquid handling steps.

Experiment 1: Enzymatic Time Course



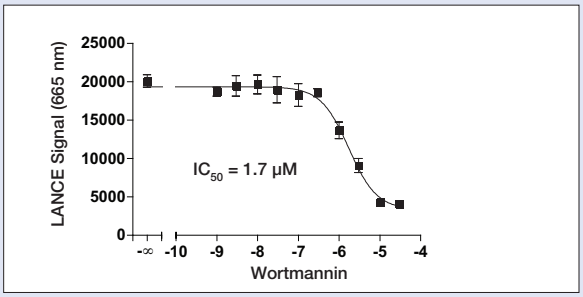
mTOR enzyme was incubated at concentrations ranging from 1.25 to 20 nM with 50 nM U*Light*-p70 S6K Peptide and 200 µM ATP. Kinase reactions were terminated after 0 to 120 min by the addition of EDTA.

Experiment 2: ATP Titration



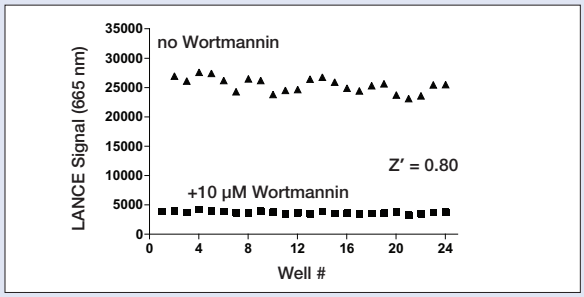
Serial dilutions of ATP ranging from 10 nM to 300 µM were added to 10 nM mTOR enzyme and 50 nM of U*Light*-p70 S6K Peptide. Kinase reactions were terminated after 60 min by the addition of EDTA.

Experiment 3: Enzyme Inhibition Curve



Serial dilutions of Wortmannin ranging from 1 nM to 30 µM (final concentrations in 1% DMSO) were incubated with 10 nM mTOR enzyme, 50 nM U*Light*-p70 S6K Peptide and 30 µM ATP. Kinase reactions were terminated after 60 min by the addition of EDTA.

Experiment 4: Z'-factor Determination



mTOR enzyme at 10 nM was incubated with 50 nM U*Light*-p70 S6K Peptide and 30 µM ATP with or without 10 µM Wortmannin (final concentrations in 1% DMSO). Kinase reactions were terminated after 60 min by the addition of EDTA.

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