TECHNICAL NOTE

# RSK2 Kinase Assay

U*Light*™ – 40S ribosomal protein S6 (pSer235/Ser236) Peptide
Europium-anti-phospho-40S ribosomal protein S6 (Ser235/236) Antibody

LANCE® *Ultra*

TECH NOTE *U*-TRF #30


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## Two LANCE *Ultra* companion products – two convenient sizes!

### U*Light* -rpS6 (pSer235/Ser236) Peptide:

- TRF0129-D: 0.5 nmole, 1,000 assay points*
- TRF0129-M: 5 nmoles, 10,000 assay points*

**0.5 pmol/assay point*

### PEPTIDE MOTIF:

RRL(pS)SLRA

Synthetic peptide containing residues surrounding Ser235 and Ser236 of human 40S ribosomal protein S6; this peptide is pre-phosphorylated on Ser235; phosphorylation site: Ser236.

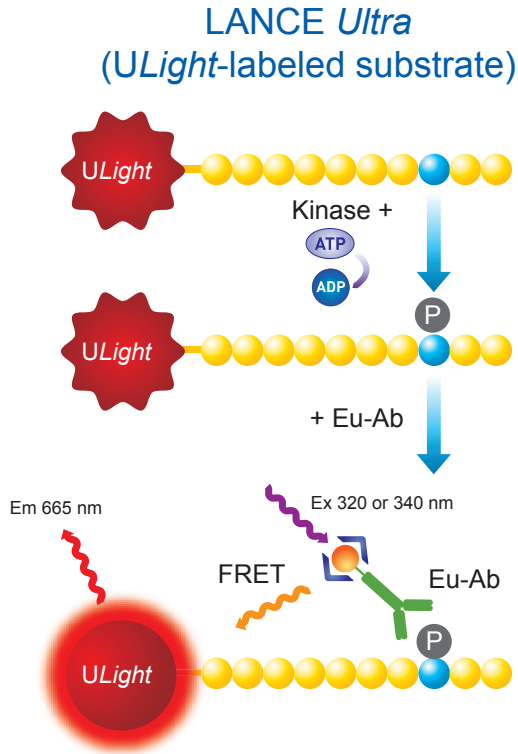
### Europium-anti-phospho-rpS6 (Ser235/236) Antibody:

- TRF0217-D: 10 µg, 1,562 assay points*
- TRF0217-M: 100 µg, 15,625 assay points*

**40 fmol/assay point*

### RECOGNIZED MOTIF:

Europium-labeled rabbit monoclonal antibody recognizing human 40S ribosomal protein S6 phosphorylated at both Ser235 and Ser236.




### LANCE *ULTRA* KINASE ASSAYS

LANCE *Ultra* time-resolved fluorescence resonance energy transfer (TR-FRET) assays use a proprietary europium chelate donor dye 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.

PerkinElmer®
*For the Better*

## Development of a RSK2 Kinase Assay

### Additional Reagents:

| | |
|---|---|
| LANCE® Detection Buffer, 10X | PerkinElmer # CR97-100 |
| OptiPlate™-384, white | PerkinElmer # 6007299 |
| TopSeal™-A | PerkinElmer # 6005185 |
| RSK2 | Carna Biosciences # 01-150 |

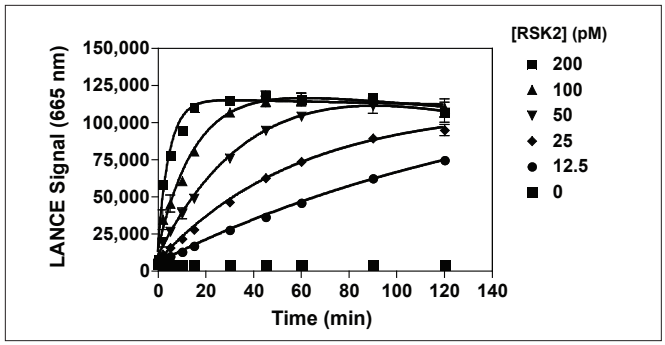
Kinase Buffer: 50 mM HEPES pH 7.5, 1 mM EGTA, 10 mM $MgCl_2$, 2 mM DTT and 0.01% Tween-20.

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

### Suggested Procedure

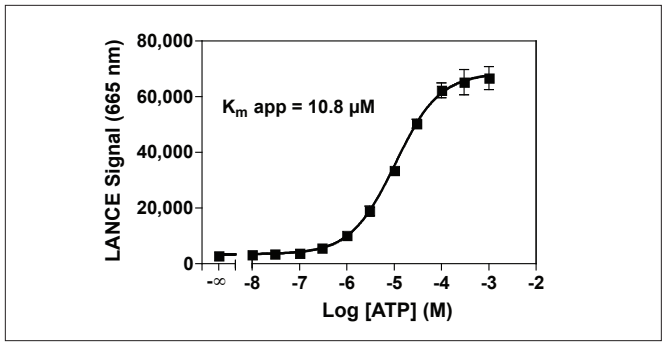
- Dilute the RSK2 enzyme, ATP, inhibitors and U*Light*-rpS6 (pSer235/Ser236) Peptide in Kinase Buffer.
- Prepare a 4X Detection Mix by diluting the Eu-anti-phospho-rpS6 (Ser235/236) Antibody to 8 nM in 1X LANCE Detection Buffer.
- Add to the wells of a white Optiplate-384:
  - 5 µL of RSK2 enzyme
  - 2.5 µL of inhibitor or Kinase Buffer
  - 2.5 µL of U*Light*-rpS6 (pSer235/Ser236) 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 24 mM EDTA prepared in 1X Detection Buffer (Stop Solution). Leave for 5 min at RT.
- Add 5 µL of Detection Mix (Eu-anti-phospho-rpS6 (Ser235/236) 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).

### Experiment 1: Enzyme Titration and Time-Course



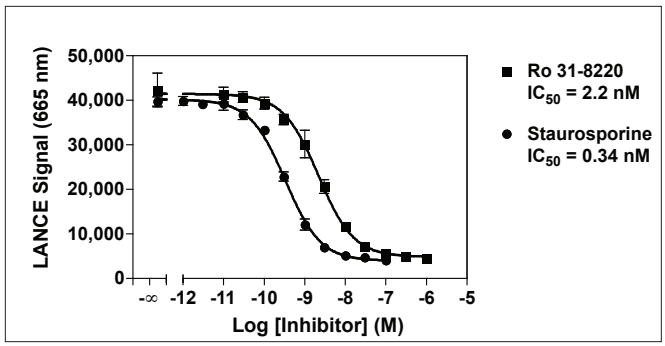

RSK2 enzyme was incubated at concentrations ranging from 12.5 to 200 pM with 50 nM U*Light*-rpS6 (pSer235/Ser236) Peptide and 100 µM ATP. Kinase reactions were terminated after 0 to 120 min by the addition of EDTA.

### Experiment 2: ATP Titration



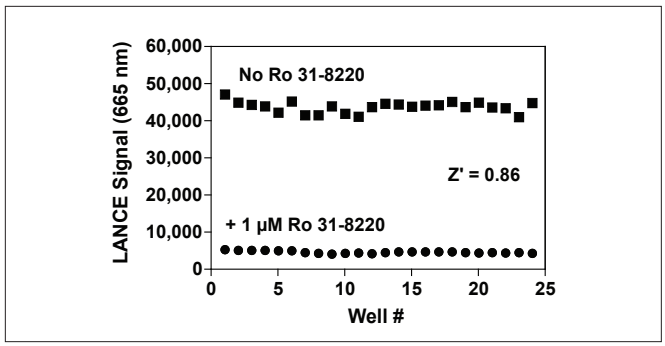

Serial dilutions of ATP ranging from 10 nM to 1 mM were added to 25 pM RSK2 enzyme and 50 nM of U*Light*-rpS6 (pSer235/Ser236) Peptide. Kinase reactions were terminated after 60 min by the addition of EDTA.

### Experiment 3: Enzyme Inhibition




Serial dilutions of Ro 31-8220 ranging from 10 pM to 1 µM and serial dilutions of staurosporine ranging from 1 pM to 100 nM (final concentrations in 2% DMSO) were incubated with 25 pM RSK2 enzyme, 50 nM U*Light*-rpS6 (pSer235/Ser236) Peptide and 10 µM ATP. Kinase reactions were terminated after 60 min by the addition of EDTA.

### Experiment 4: Z'-factor Determination




RSK2 enzyme at 25 pM was incubated with 50 nM U*Light*-rpS6 (pSer235/Ser235) Peptide and 10 µM ATP with or without 1 µM Ro 31-8220 (final concentrations in 2% DMSO). Kinase reactions were terminated after 60 min by the addition of EDTA.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