TECHNICAL DATA SHEET

THUNDER™ Phospho-RPS6 (S235/S236) + Total RPS6 TR-FRET Cell Signaling Assay Kit

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CATALOG NUMBERS KIT-RPS6APT-500
400 points for phospho-RPS6 and 100 points for total RPS6

Store at -80°C
For research use only.
Not for use in diagnostic procedures.

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PRODUCT DESCRIPTION

This assay kit measures intracellular levels of **phospho-RPS6 (S235/S236)** and **total RPS6** protein in cell lysates using a simple, rapid and sensitive immunoassay based on the homogeneous (no-wash) THUNDER™ TR-FRET technology. The kit is compatible with both adherent and suspension cells.

SPECIFICITY

This assay kit contains two specific and selective antibody pairs, one that recognizes **RPS6** phosphorylated at **Ser235** and **Ser236** and another that recognizes **total** (both phosphorylated and unphospho-rylated) **RPS6.**

SPECIES REACTIVITY

Human (Swiss-Prot Acc.: P62753; Entrez-Gene Id: 6194).

Other species should be tested on a case-by-case basis.

TR-FRET ASSAY PRINCIPLE

The **Phospho-RPS6 (S235/S236) + Total RPS6** assay kit is a homogeneous time-resolved Förster resonance energy transfer (TR-FRET) sandwich immunoassay (Figure 1). The THUNDER™ Cell Signaling assay workflow consists of 3 steps (Figure 2). Following cell treatment, cells are first lysed with the specific Lysis Buffer provided in the kit. Then **Phospho-RPS6 (S235/S236)** and **Total RPS6** in the cell lysates are detected in separate wells with two pairs of fluorophore-labeled antibodies in a simple "add-incubate-measure" format (single-step reagent addition; no wash steps). For detection of the phosphorylated protein, one antibody is labeled with a donor fluorophore (Europium chelate; Eu-Ab1) and the second with a far-red acceptor fluorophore (FR-Ab2). The same approach is used for the second antibody pair detecting the total protein (Eu-Ab3 and FR-Ab4). The binding of the two matched labeled antibodies to distinct epitopes on the target protein (either **phospho-RPS6** or **total RPS6**) takes place in solution and brings the two dyes into close proximity. Excitation of the donor Europium chelate molecules with a flash lamp (320 or 340 nm) or a laser (337 nm) triggers a FRET from the donor to the acceptor molecules, which in turn emit a TR-FRET signal at 665 nm. Residual energy from the Eu chelate generates light at 615 nm. The signal at 665 nm is proportional to the concentration of **Phospho-RPS6 (S235/S236)** and **Total RPS6** in the cell lysate. Data can be expressed as either the signal at 665 nm or the 665 nm/615 nm ratio.

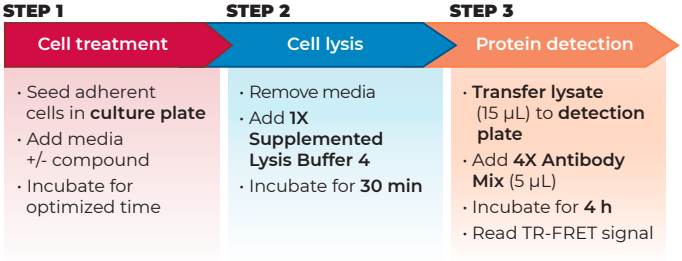

Figure 2 Assay workflow using the 2-plate (transfer) protocol.

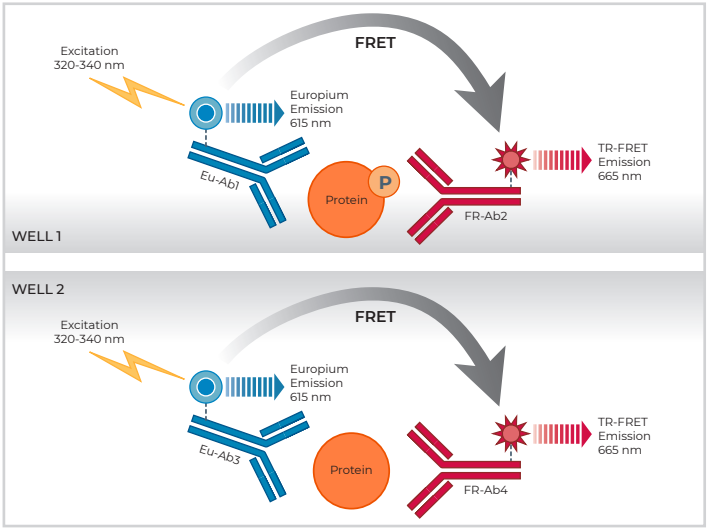

Fig. 1 Schematic representation of the TR-FRET cell signaling assay principle.

KIT COMPONENTS

KIT COMPONENTS	500 points*
Eu-labeled phospho-RPS6 (S235/S236) antibody (Eu-Ab1)	20 µL
Acceptor-labeled phospho-RPS6 (S235/S236) antibody (FR-Ab2)	80 µL
Eu-labeled total-RPS6 antibody (Eu-Ab3)	5 µL
Acceptor-labeled total-RPS6 antibody (FR-Ab4)	20 µL
Lysis Buffer 4 (5X)	5 mL
Detection Buffer (10X)	250 µL
Positive control cell lysate	200 µL
Phosphatase Inhibitor Cocktail (100X)	250 µL

* The number of assay points is based on an assay volume of 20 µL in half-area 96-well or low-volume 384-well assay plates using the kit components at the recommended concentrations (refer to the User Manual).

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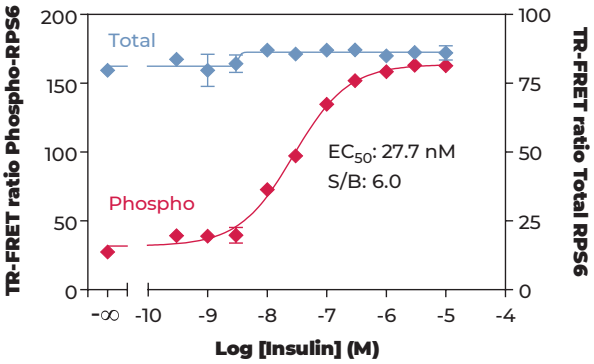
Phospho-RPS6 (S235/S236) + Total RPS6

VALIDATION DATA

This assay kit has been validated for the relative quantification of phospho-RPS6 (S235/S236) and total RPS6 in MCF7 cell lysates using the 2-plate assay protocol.

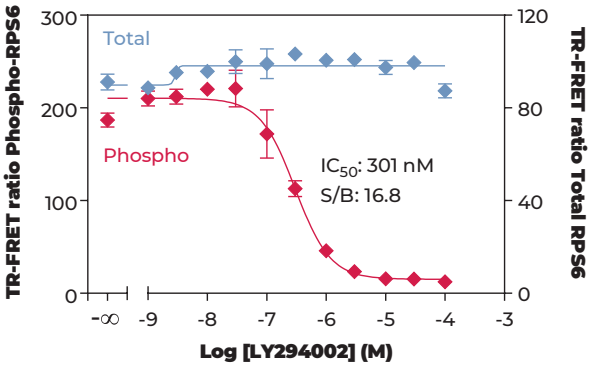
- Adherent cells were cultured overnight in a 96-well tissue culture plate (EMEM +10% FBS) and then serum starved for 18 hours.
- Following cell treatment, the media was removed and cells were lysed with the 1X **Lysis Buffer 4** (50 µL) supplemented with the phosphatase inhibitors sodium fluoride (1 mM) and sodium orthovanadate (2 mM).
- Following a **30-min** incubation at room temperature (RT) on an orbital shaker (400 rpm), lysates (15 µL) were then transferred to a 384-well assay plate followed by addition to separate wells of either the labeled antibodies Eu-Ab1 and FR-Ab2 (5 µL) for detection of phospho-RPS6 (S235/S236) or Eu-Ab3 and FR-Ab4 (5 µL) for detection of total RPS6.
- The plate was incubated at RT for **4 hours** and the TR-FRET signal was recorded at 665 and 615 nm (EnVision®; lamp excitation).

STIMULATION OF PHOSPHO-RPS6 (S235/S236) IN MCF7 CELLS



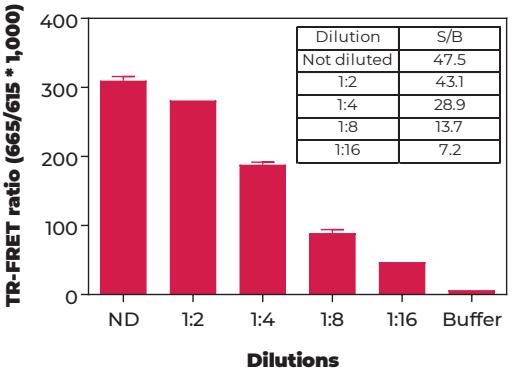
MCF7 cells (75,000 cells/well; in triplicate) were incubated with serial dilutions of insulin for 60 min at RT. Data show that treatment of MCF7 cells with insulin stimulates phosphorylation of RPS6 at S235/S236, but does not affect the levels of total RPS6.

INHIBITION OF PHOSPHO-RPS6 (S235/S236) IN MCF7 CELLS



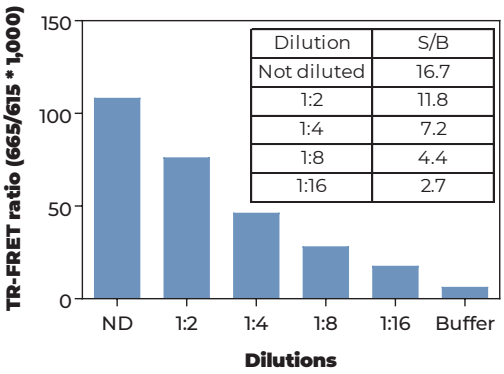
MCF7 cells (75,000 cells/well; in triplicate) were incubated with serial dilutions of the inhibitor LY294002 for 60 min at RT. Cells were then stimulated with 1 µM of insulin for 60 min at RT. Data show that treatment of MCF7 cells with LY294002 inhibits phosphorylation of RPS6 at S235/S236 by insulin, but does not affect the levels of total RPS6.

MCF7 CONTROL LYSATE TITRATION (QC TEST) PHOSPHO-RPS6 (S235/S236)



Dilution	S/B
Not diluted	47.5
1:2	43.1
1:4	28.9
1:8	13.7
1:16	7.2

MCF7 CONTROL LYSATE TITRATION (QC TEST) TOTAL RPS6



Dilution	S/B
Not diluted	16.7
1:2	11.8
1:4	7.2
1:8	4.4
1:16	2.7

Quality Control: the Phospho-RPS6 (S235/S236) + Total RPS6 assay kit is routinely tested against Insulin-treated MCF7 lysates. MCF7 cells were cultured in a T175 flask to 90% confluence and stimulated with 10 µM of insulin for 60 min at RT. Following cell lysis using 16 mL of 1X Lysis Buffer 4, lysates were serially diluted with 1X Lysis Buffer 4 and tested in triplicate and in separate wells for phospho-RPS6 (S235/S236) and total RPS6. Data show a linear relationship between lysate dilutions and TR-FRET ratio values.

FOR MORE INFORMATION ON DEVELOPING AND OPTIMIZING TR-FRET CELL SIGNALING ASSAYS, CONSULT THE USER MANUAL.

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7171 Frederick-Banting, Suite 2115, Montréal (Québec) H4S 1Z9 CANADA **bioauxilium.com** **1-866-610-6933 x 103**