TECHNICAL DATA SHEET

THUNDER™ Phospho-eIF4E (S209) + Total eIF4E
TR-FRET Cell Signaling Assay Kit

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CATALOG NUMBERS KIT-EIF4EPT-500
400 points for phospho-eIF4E and 100 points for total eIF4E

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-eIF4E (S209)** and **total eIF4E** 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 **eIF4E** phosphorylated at **Ser209** and another that recognizes **total** (both phosphorylated and unphospho-rylated) **eIF4E.**

SPECIES REACTIVITY

Human (Swiss-Prot Acc. P06730 and Entrez-Gene Id 1977).

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

TR-FRET ASSAY PRINCIPLE

The **Phospho-eIF4E (S209) + Total eIF4E** assay kit is a homo-geneous 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-eIF4E (S209)** and **Total eIF4E** 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-eIF4E** or **total eIF4E**) 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-eIF4E (S209)** and **Total eIF4E** in the cell lysate. Data can be expressed as either the signal at 665 nm or the 665 nm/615 nm ratio.

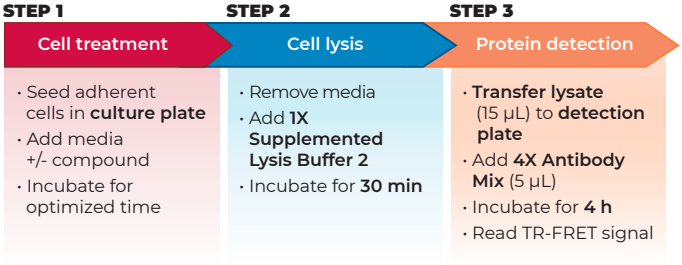

Figure 2 Assay workflow using the 2-plate (transfer) protocol.

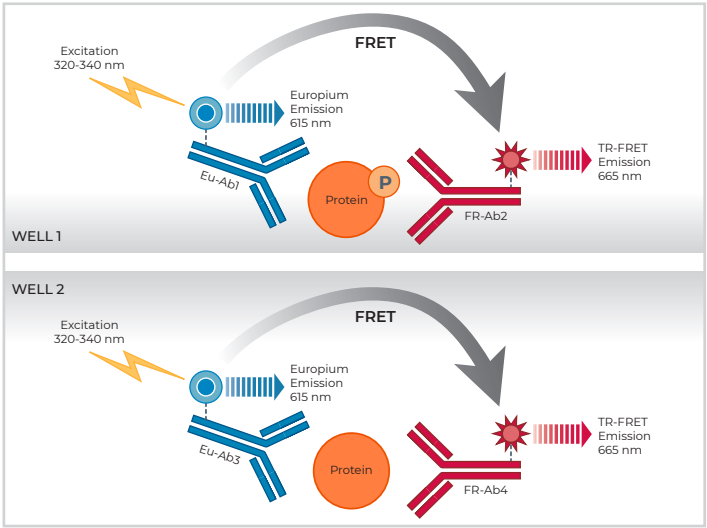

Fig. 1 Schematic representation of the TR-FRET cell signaling assay principle.

KIT COMPONENTS

	500 points*
Eu-labeled phospho-eIF4E (S209) antibody (Eu-Ab1)	20 µL
Acceptor-labeled phospho-eIF4E (S209) antibody (FR-Ab2)	80 µL
Eu-labeled total-eIF4E antibody (Eu-Ab3)	5 µL
Acceptor-labeled total-eIF4E antibody (FR-Ab4)	20 µL
Lysis Buffer 2 (5X)	5 mL
Detection Buffer (10X)	250 µL
Positive control cell lysate	500 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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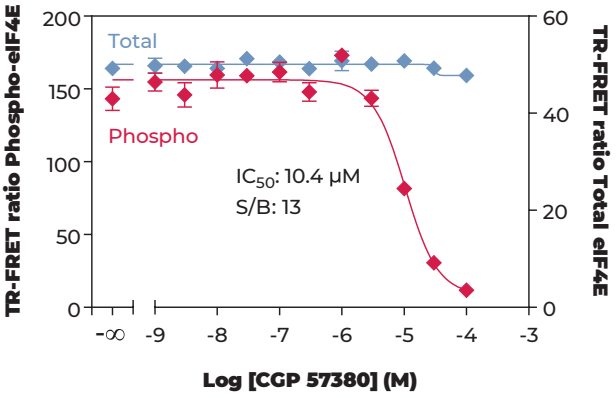
Phospho-eIF4E (S209) + Total eIF4E

VALIDATION DATA

This assay kit has been validated for the relative quantification of phospho-eIF4E (S209) and total eIF4E in HEK293 cell lysates using the 2-plate assay protocol.

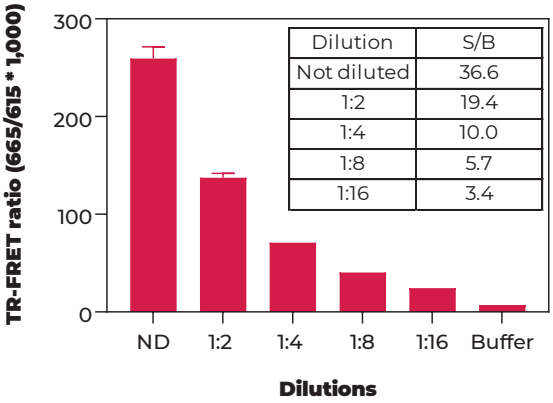
- Adherent cells were cultured overnight in a 96-well tissue culture plate coated with poly-L-lysine (EMEM +10% FBS).
- Following cell treatment, the media was removed and cells were lysed with the 1X **Lysis Buffer 2** (50 µL) supplemented with the phosphatase inhibitors sodium fluoride (1 mM) and sodium 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-eIF4E (S209) or Eu-Ab3 and FR-Ab4 (5 µL) for detection of total eIF4E.
- The plate was incubated at RT for **4 hours** and the TR-FRET signal was recorded at 665 and 615 nm (EnVision®; lamp excitation).

INHIBITION OF PHOSPHO-eIF4E (S209) IN HEK293 CELLS



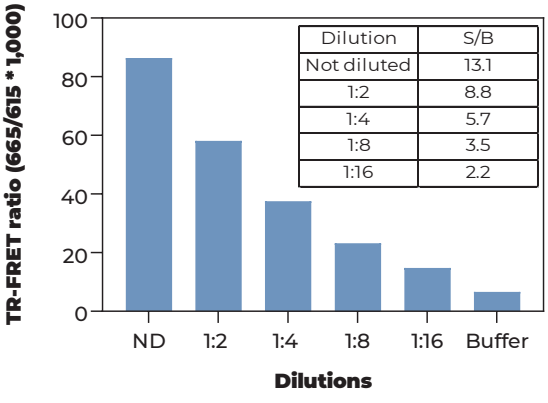
HEK293 cells (50,000 cells/well; in triplicate) were incubated with serial dilutions of the inhibitor CGP 57380 for 30 min at 37°C. Cells were then stimulated with 10 nM of EGF for 30 min at 37°C. Data show that treatment of HEK293 cells with CGP 57380 inhibits phosphorylation of eIF4E at S209 by EGF, but does not affect the levels of total eIF4E.

HEK293 CONTROL LYSATE TITRATION (QC TEST) PHOSPHO-eIF4E (S209)



Dilution	S/B
Not diluted	36.6
1:2	19.4
1:4	10.0
1:8	5.7
1:16	3.4

HEK293 CONTROL LYSATE TITRATION (QC TEST) TOTAL eIF4E



Dilution	S/B
Not diluted	13.1
1:2	8.8
1:4	5.7
1:8	3.5
1:16	2.2

Quality Control: the Phospho-eIF4E (S209) + Total eIF4E assay kit is routinely tested against EGF-treated HEK293 lysates. HEK293 cells were cultured in a T175 flask to 85% confluence and stimulated with 30 nM EGF for 30 min at 37°C. Following cell lysis using 8 mL of 1X Lysis Buffer 2, lysates were serially diluted with 1X Lysis Buffer 2 and tested in triplicate and in separate wells for phospho-eIF4E (S209) and total eIF4E. 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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