TECHNICAL DATA SHEET

THUNDER™ Phospho-RIPK1 (S166) + Total RIPK1 TR-FRET Cell Signaling Assay Kit

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CATALOG NUMBERS KIT-RIPK1PT-500
400 points for phospho-RIPK1 and 100 points for total RIPK1

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

Visit Product Page

PRODUCT DESCRIPTION

This assay kit measures intracellular levels of **phospho-RIPK1 (S166) and total RIPK1** 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 **RIPK1** phosphorylated at **Ser166**, and another that recognizes total (both phosphorylated and unphosphorylated) RIPK1.

SPECIES REACTIVITY

Human (Swiss-Prot Acc.: Q13546; Entrez-Gene Id: 8737).

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

TR-FRET ASSAY PRINCIPLE

The **Phospho-RIPK1 (S166) + Total RIPK1** 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-RIPK1 (S166) + Total RIPK1** in the cell lysates is detected with a pair of fluorophore-labeled antibodies in a simple "add-incubate-measure" format (single-step reagent addition; no wash steps). One antibody is labeled with a donor fluorophore (Europium chelate; Eu-Ab1) and the second with a far-red acceptor fluorophore (FR-Ab2). The binding of the two matched labeled antibodies to distinct epitopes on the target protein (either **phospho-RIPK1** or **total RIPK1**) 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-RIPK1 (S166)** and **Total RIPK1** in the cell lysate. Data can be expressed as either the signal at 665 nm or the 665 nm/615 nm rationm/615 nm ratio.

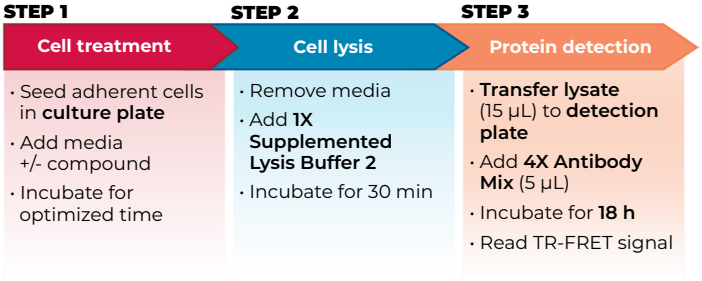


Figure 2 Assay workflow using the 2-plate (transfer) protocol.

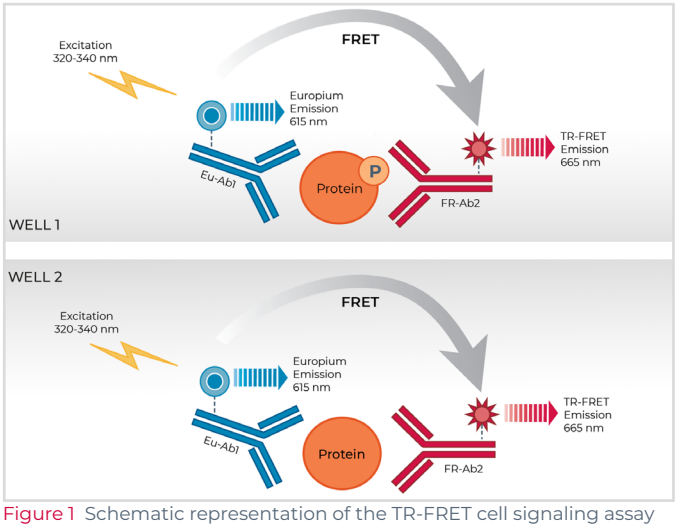


Figure 1 Schematic representation of the TR-FRET cell signaling assay principle.

KIT COMPONENTS

	500 points*
Eu-labeled phospho-RIPK1 (S166) antibody (Eu-Ab1)	20 µL
Acceptor-labeled phospho-RIPK1 (S166) antibody (FR-Ab2)	80 µL
Eu-labeled total-RIPK1 antibody (Eu-Ab3)	5 µL
Acceptor-labeled total-RIPK1 antibody (FR-Ab4)	20 µL
Lysis Buffer 2 (5X)	5 mL
Detection Buffer (10X)	250 µL
Positive control cell lysate	200 µL
Phosphatase Inhibitor Cocktail (100X)	250 µL

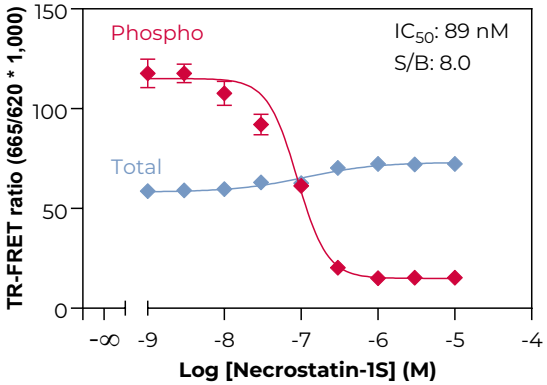
* The number of assay points is based on an assay volume of 20 µL in half-area 96-well or low-volume 384-well assay plates using the kit components at the recommended concentrations (refer to the User Manual).

VALIDATION DATA

This assay kit has been validated for the relative quantification of phospho-RIPK1 (S166) in HT-29 cell lysates using the 2-plate assay protocol.

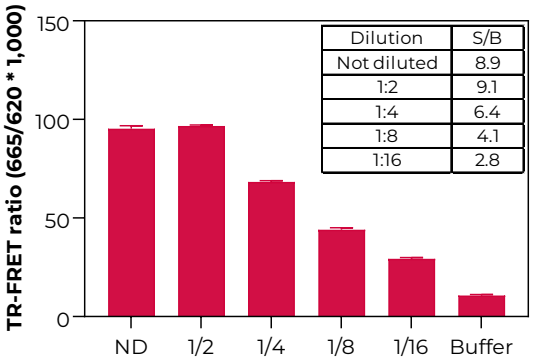
- Adherent cells were cultured overnight in a 96-well tissue culture plate (McCoy's 5A + 10% FBS).
- Following cell treatment, the media was removed and cells were lysed with the 1X Lysis Buffer 2 (50 µL) supplemented with the 100X Phosphatase Inhibitor Cocktail diluted at 1X.
- Following a **30-min** 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 of the labeled antibodies Eu-Ab1 and FR-Ab2 (5 µL) for detection of phospho-RIPK1 (S166) or Eu-Ab3 and FR-Ab4 (5 µL) for detection of total RIPK1.
- The plate was incubated at RT for **18 hours** and the TR-FRET signal was recorded at 665 and 615 nm (PHERASTAR® FSX; laser excitation).

INHIBITION OF PHOSPHO-RIPK1 (S166) IN HT-29 CELLS



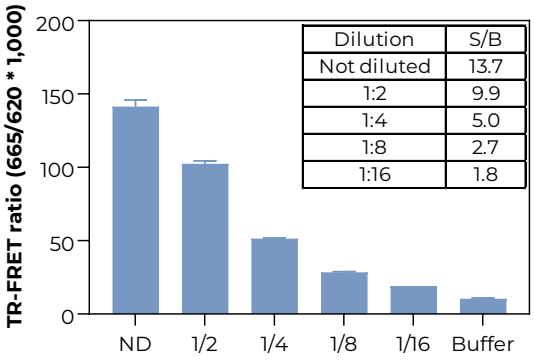
HT-29 cells (100,000 cells/well; in triplicate) were pre-incubated 20 minutes with 25 µM of Z-VAD(OMe)-FMK at 37°C. Serial dilutions of Necrostatin-1S were then added along with SM-164 at a final concentration of 100 nM; cells were incubated for an additional 10 minutes at 37°C. Finally, TNFα was added at a final concentration of 0.17 pM and cells were incubated for an additional 6 hours at 37°C. Data show that treatment of HT-29 cells with Necrostatin-1S inhibits phosphorylation of RIPK1 at S166 by TNFα.

HT-29 CONTROL LYSATE TITRATION (QC TEST) PHOSPHO-RIPK1 (S166)



Dilution	S/B
Not diluted	8.9
1:2	9.1
1:4	6.4
1:8	4.1
1:16	2.8

HT-29 CONTROL LYSATE TITRATION (QC TEST) TOTAL RIPK1



Dilution	S/B
Not diluted	13.7
1:2	9.9
1:4	5.0
1:8	2.7
1:16	1.8

Quality Control: the Phospho-RIPK1 (S166) and Total RIPK1 assay kit is routinely tested against TNFα treated HT-29 lysates. HT-29 cells were cultured in a T175 flask to 100% confluency and incubated in 25 µM of Z-VAD(OMe)-FMK for 20 min at 37°C before adding SM-164 at a final concentration of 100 nM. After an additional 10 min of incubation at 37°C, the cells were stimulated with 0.11 pM of TNFα for 6 hours at 37°C. Following cell lysis using 5 mL of 1X Lysis Buffer 2 supplemented with Phosphatase Inhibitor Cocktail, lysates were serially diluted with 1X Lysis Buffer 2 and tested in triplicate. 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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