TECHNICAL DATA SHEET

THUNDER™ Phospho-SLP-76 (S376) TR-FRET Cell Signaling Assay Kit

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CATALOG NUMBERS

KIT-SLP76P-100 (100 tests)
KIT-SLP76P-500 (500 tests)
KIT-SLP76P-2500 (2500 tests)
KIT-SLP76P-5000 (5000 tests)
KIT-SLP76P-10000 (10000 tests)

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

PRODUCT DESCRIPTION

This assay kit measures intracellular levels of **phospho-SLP-76 (S376)** 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 antibodies, one that recognizes **SLP-76** phosphorylated at **Ser376** and another that recognizes an invariant epitope of **SLP-76**.

SPECIES REACTIVITY

Human (Swiss-Prot Acc.: Q13094; Entrez-Gene Id: 3937).

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

TR-FRET ASSAY PRINCIPLE

The **Phospho-SLP-76 (S376)** 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-SLP-76 (S376)** 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 labeled antibodies to distinct epitopes on the target protein 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-SLP-76 (S376)** in the cell lysate. Data can be expressed as either the signal at 665 nm or the 665 nm/615 nm ratio.

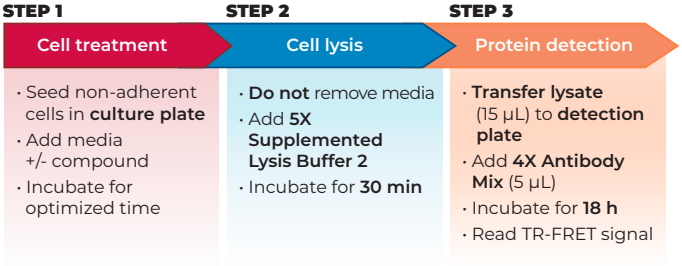


Figure 2 Assay workflow using the 2-plate (transfer) protocol.

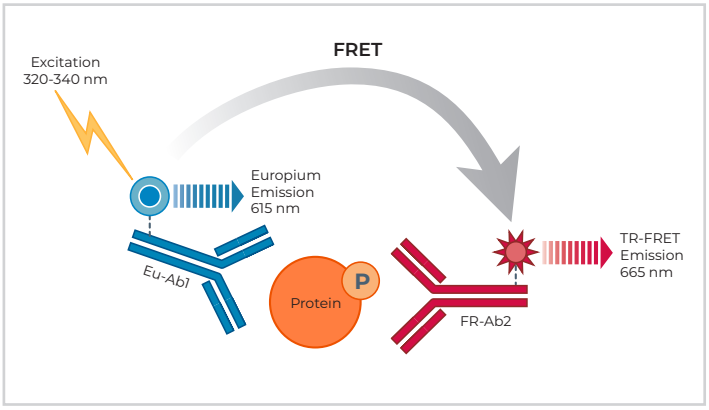


Figure 1 Schematic representation of the TR-FRET cell signaling assay principle.

KIT COMPONENTS

	100 points*	500 points*
Eu-labeled phospho-SLP-76 (S376) antibody (Eu-Ab1)	5 µL	25 µL
Acceptor-labeled phospho-SLP-76 (S376) antibody (FR-Ab2)	20 µL	100 µL
Lysis Buffer 2 (5X)	1 mL	5 mL
Detection Buffer (10X)	50 µL	250 µL
Positive control cell lysate	100 µL	500 µL
Phosphatase Inhibitor Cocktail (100X)	50 µL	250 µL

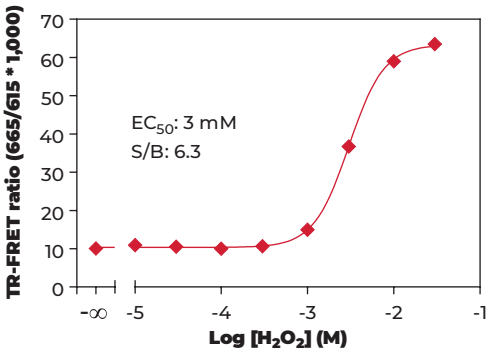
* The number of assay points is based on an assay volume of 20 µL in half-area 96-well or low-volume 384-well assay plates using the kit components at the recommended concentrations (refer to the User Manual).

VALIDATION DATA

This assay kit has been validated for the relative quantification of phospho-SLP-76 (S376) in Jurkat cell lysates using the 2 plate assay protocol.

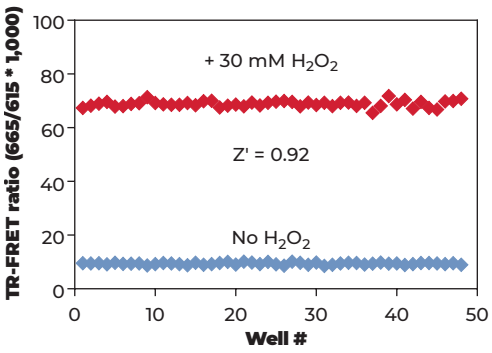
- Non-adherent cells were cultured in RPMI+10% FBS before being centrifuged and resuspended at the desired density in RPMI without serum.
- Following cell treatment, cells were lysed with the 5X **Lysis Buffer 2** supplemented with the 100X Phosphatase Inhibitor Cocktail diluted at 5X.
- Following a **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-SLP-76 (S376).
- The plate was incubated at RT for **18 hours** and the TR-FRET signal was recorded at 665 and 615 nm (EnVision®; lamp excitation).

STIMULATION OF PHOSPHO-SLP-76 (S376) IN JURKAT CELLS



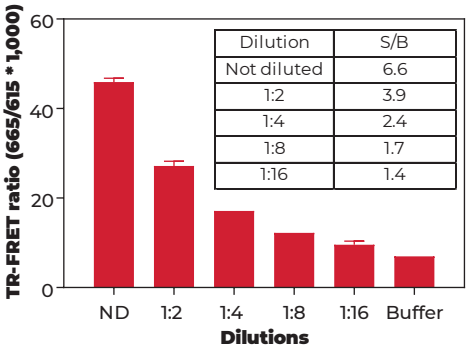
Jurkat cells (400,000 cells/well in triplicate) plated in a 96-well tissue culture plate were incubated with serial dilutions of H_2O_2 for 15 min at RT. Data show that treatment of Jurkat cells with H_2O_2 stimulates phosphorylation of SLP-76 at S376.

Z'-FACTOR DETERMINATION IN JURKAT CELLS



Jurkat cells (400,000 cells/well) were incubated without or with 30 mM H_2O_2 for 15 min at RT. The Z' factor value was determined using a total of 48 wells for each treatment group. The Z'-factor value of 0.92 indicates that the assay is robust and suitable for HTS.

JURKAT CONTROL LYSATE TITRATION (QC TEST)



Dilution	S/B
Not diluted	6.6
1:2	3.9
1:4	2.4
1:8	1.7
1:16	1.4

Quality Control: the Phospho-SLP-76 (S376) assay kit is routinely tested against H_2O_2 treated Jurkat lysates. Jurkat cells were cultured, centrifuged, resuspended at 20 million cells/mL and stimulated with 30 mM of H_2O_2 for 15 min at RT. Following cell lysis with 5X Lysis Buffer 2 (final concentration: 1X), 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.

ALL-IN-ONE-WELL PHOSPHO-SLP-76 (S376) ASSAY

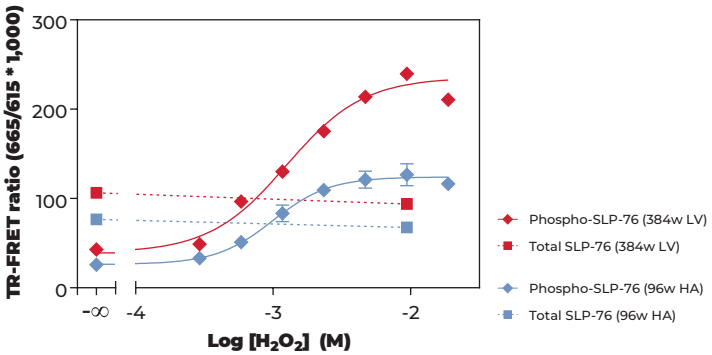


Plate	384-well LV	96-well HA
EC50 (mM)	1.2	1.0
S/B	5.6	4.9

Jurkat cells (100,000 cells/well in triplicate) were plated in 8 µL in either a low volume (LV) 384-well assay plate or a half-area (HA) 96-well assay plate and immediately incubated with 4 µL of H_2O_2 for 15 min at RT. Cells were then lysed with 3 µL of 5X supplemented Lysis Buffer 2. Following a 30-min incubation at RT on an orbital shaker (400 rpm), 5 µL of labeled antibodies Eu-Ab1 and FR-Ab2 were added directly to the lysate for detection of phospho-SLP-76 (S376) in the same well, without a transfer step (1-plate assay protocol). Data show that stimulation of phospho-SLP-76 (S376) by H_2O_2 in Jurkat cells can be detected using the 1-plate assay protocol in both LV 384 and HA 96-well plates.

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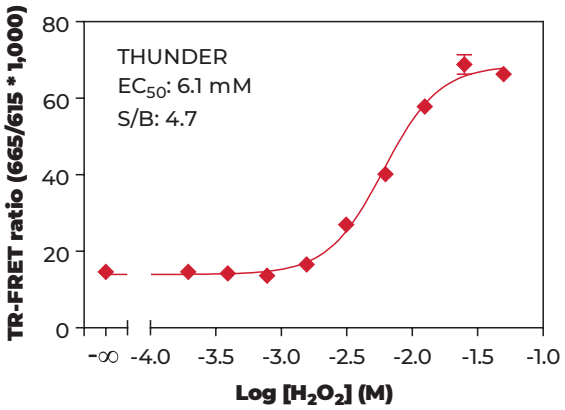
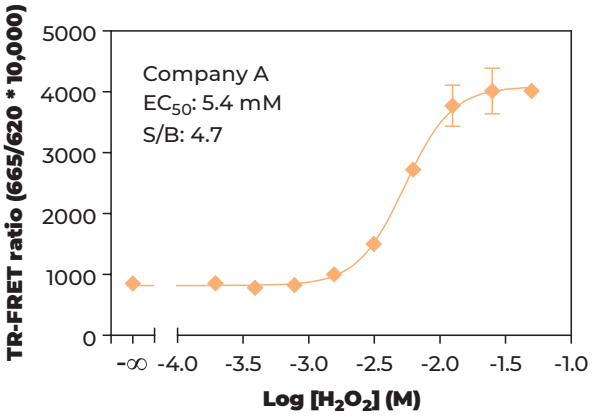
KIT BENCHMARKING – COMPARISON TO OTHER TR-FRET TECHNOLOGIES

As part of assay validation, the THUNDER™ Phospho-SLP-76 (S376) Assay Kit was benchmarked against two competitive TR-FRET assay technologies (Companies A and B). Specifically, the 3 different assays were compared side-by-side for their capacity to detect phospho-SLP-76 (S376) stimulation upon Jurkat cell treatment with H_2O_2 using the 2-plate assay protocol. All reagents were prepared according to each manufacturer's recommendations. Following cell treatment, cells were lysed with the corresponding kit's 1X Lysis Buffer supplemented with phosphatase inhibitors. Lysates were then tested on the same 384-well assay plate and according to the corresponding kit's standard protocol. The plate was read on an EnVision® (lamp excitation) following 4 hours (THUNDER™ and Company B) or 18 hours of incubation (Company A). Data show that the 3 TR-FRET assays exhibited comparable sensitivity (EC_{50} values). The Company B assay showed a slightly higher S/B ratio.

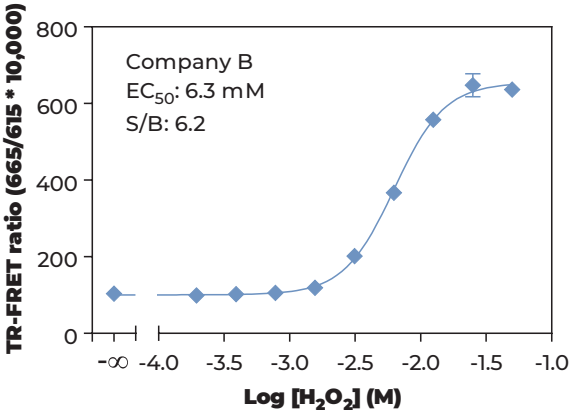
THUNDER™ PHOSPHO-SLP-76 (S376) ASSAY KIT



COMPANY A PHOSPHO-SLP-76 (S376) ASSAY KIT



COMPANY B PHOSPHO-SLP-76 (S376) ASSAY KIT



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