TECHNICAL DATA SHEET

THUNDER™ Phospho-SRC (Y419) TR-FRET Cell Signaling Assay Kit

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CATALOG NUMBERS

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

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-SRC (Y419)** 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 **SRC** phosphorylated at **Tyr419** and another that recognizes an invariant epitope of **SRC**.

SPECIES REACTIVITY

Human (Swiss-Prot Acc.: P12931; Entrez-Gene Id: 6714).

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

TR-FRET ASSAY PRINCIPLE

The **Phospho-SRC (Y419)** 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-SRC (Y419)** 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-SRC (Y419)** in the cell lysate. Data can be expressed as either the signal at 665 nm or the 665 nm/615 nm ratio.

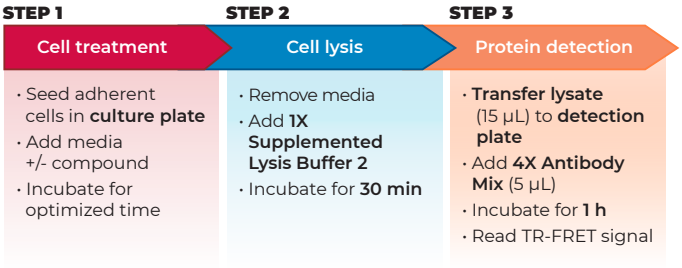

Figure 2 Assay workflow using the 2-plate (transfer) protocol.

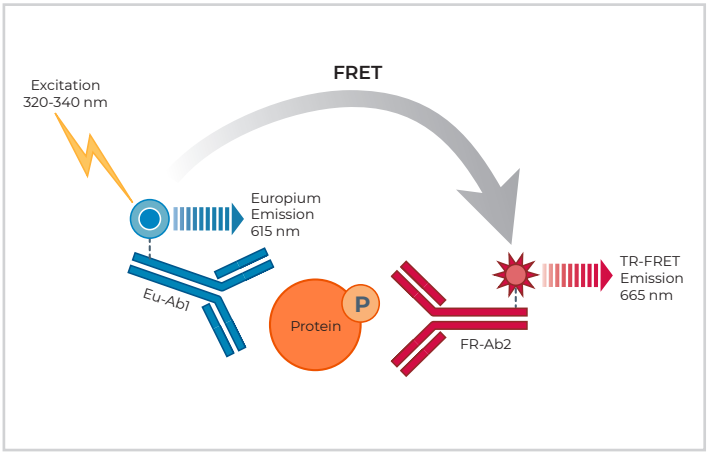

Figure 1 Schematic representation of the TR-FRET cell signaling assay principle.

KIT COMPONENTS

	100 points*	500 points*
Eu-labeled phospho-SRC (Y419) antibody (Eu-Ab1)	5 µL	25 µL
Acceptor-labeled phospho-SRC (Y419) 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	200 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).

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TECHNICAL DATA SHEET

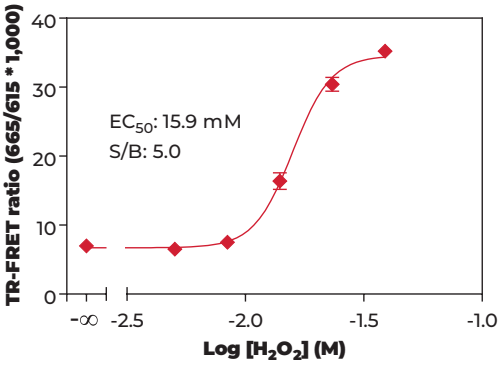
Phospho-SRC (Y419)

VALIDATION DATA

This assay kit has been validated for the relative quantification of phospho-SRC (Y419) in A431 and A549 cell lysates using the 2-plate assay protocol.

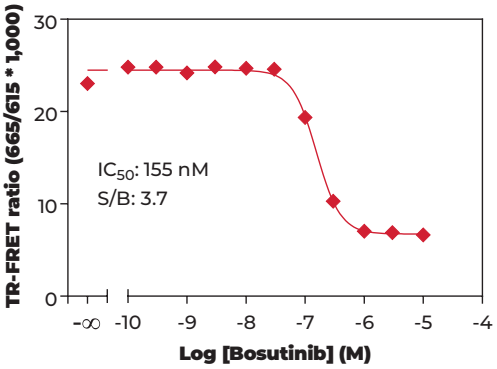
- Adherent cells were cultured overnight in a 96-well tissue culture plate (DMEM +10% FBS).
- Following cell treatment, the media was removed and cells were lysed with the 1X **Lysis Buffer 2** (50 µL) supplemented with the phosphatase inhibitors sodium fluoride (1 mM) and sodium orthovanadate (2 mM).
- 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 of the labeled antibodies Eu-Ab1 and FR-Ab2 (5 µL) for detection of phospho-SRC (Y419).
- The plate was incubated at RT for **1 hour** and the TR-FRET signal was recorded at 665 and 615 nm (EnVision®; lamp excitation).

STIMULATION OF PHOSPHO-SRC (Y419) IN A431 CELLS



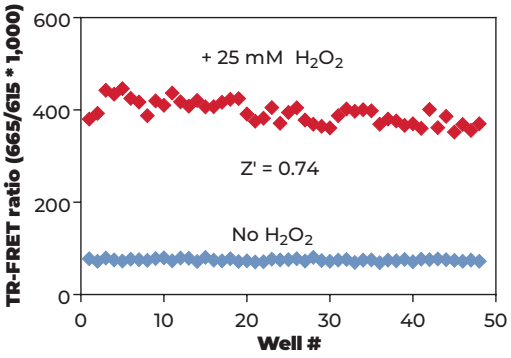
A431 cells (40,000 cells/well; in triplicate) were incubated with serial dilutions of H_2O_2 for 10 min at RT. Data show that treatment of A431 cells with H_2O_2 stimulates phosphorylation of SRC at Y419.

INHIBITION OF PHOSPHO-SRC (Y419) IN A431 CELLS



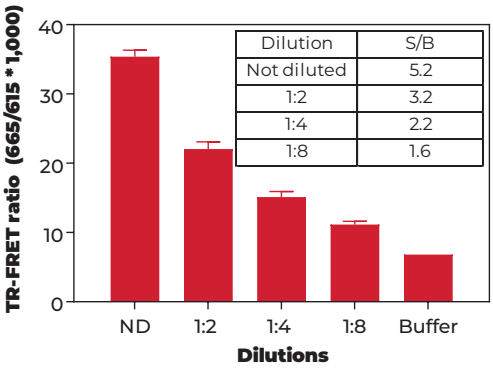
A431 cells (40,000 cells/well; in triplicate) were incubated with serial dilutions of the inhibitor Bosutinib for 10 min at RT. Cells were then stimulated with 20 mM of H_2O_2 for 10 min at RT. Data show that treatment of A431 cells with Bosutinib inhibits phosphorylation of SRC at Y419 by H_2O_2.

Z'-FACTOR DETERMINATION IN A431 CELLS



A431 cells (40,000 cells/well) were incubated without or with 25 mM H_2O_2 for 10 min at RT. The Z' factor value was determined using a total of 48 wells for each treatment group. The Z'-factor value of 0.74 indicates that the assay is robust and suitable for HTS.

A431 CONTROL LYSATE TITRATION (QC TEST)



Quality Control: the Phospho-SRC (Y419) assay kit is routinely tested against H_2O_2-treated A431 lysates. A431 were cultured in a T175 flask to 90% confluence and stimulated with 30 mM of H_2O_2 for 10 min at RT. Following cell lysis using 4 mL of 1X Lysis Buffer 2, 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.

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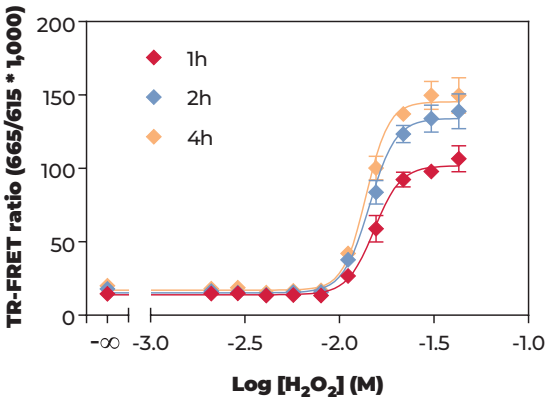
STIMULATION OF PHOSPHO-SRC (Y419) IN A549 CELLS



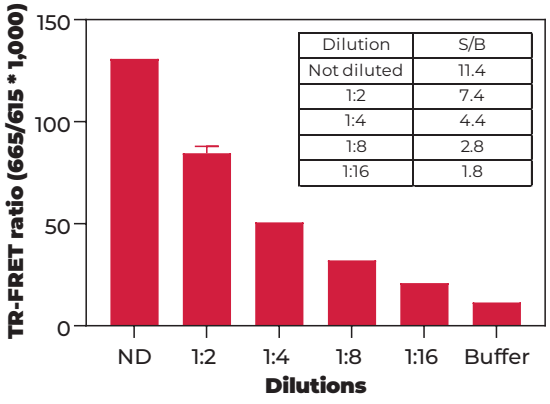
A549 cells (50,000 cells/well; in triplicate) were cultured overnight in a 96-well tissue culture plate. Cells were then incubated with serial dilutions of H_2O_2 for 10 min at RT. Data show that treatment of A549 cells with H_2O_2 stimulates phosphorylation of SRC at Y419.

Incubation time	1 h	2 h	4 h
EC_{50} (mM)	15.2	14.4	13.8
S/B	7.3	7.8	7.4

KIT BENCHMARKING – COMPARISON TO OTHER TR-FRET TECHNOLOGIES

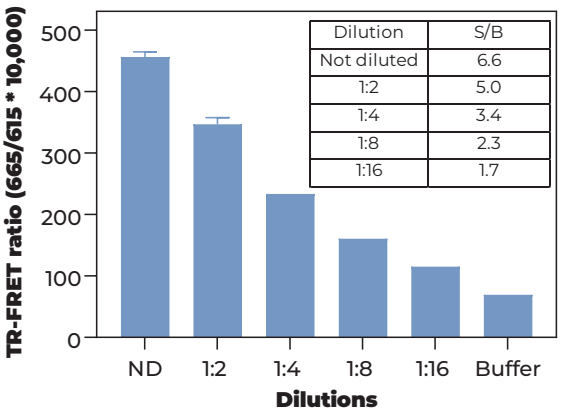
As part of assay validation, the THUNDER™ Phospho-SRC (Y419) Assay Kit was benchmarked against a competitive TR-FRET assay technology (Company B). Specifically, the 2 different assays were compared side-by-side for their capacity to detect phospho-SRC (Y419) stimulation upon A549 cell treatment with 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 supplemented Lysis Buffer. Lysates were then serially diluted with the corresponding 1X Lysis Buffer and 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 1 hour of incubation. Data show that the THUNDER™ TR-FRET assay exhibited a higher S/B ratio.

THUNDER™ PHOSPHO-SRC (Y419) ASSAY KIT



Dilution	S/B
Not diluted	11.4
1:2	7.4
1:4	4.4
1:8	2.8
1:16	1.8

COMPANY B PHOSPHO-SRC (Y419) ASSAY KIT



Dilution	S/B
Not diluted	6.6
1:2	5.0
1:4	3.4
1:8	2.3
1:16	1.7

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

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