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TECHNICAL DATA SHEET

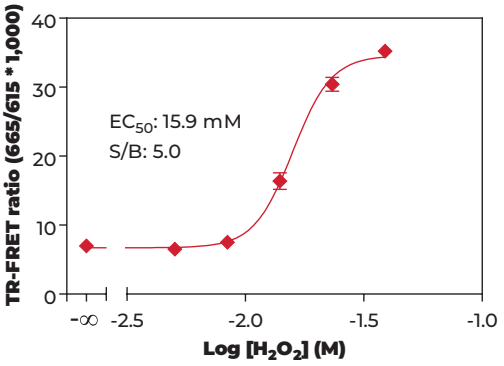
Phospho-SRC (Y419)

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.

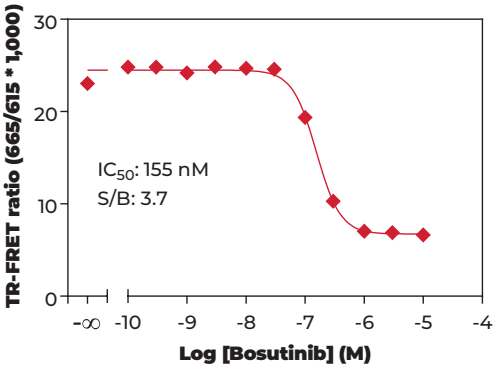
- Adherent cells were cultured overnight in a 96-well tissue culture plate (DMEM +10% FBS).
- Following cell treatment, the media was removed and cells were lysed with the 1X **Lysis Buffer 2** (50 µL) supplemented with the phosphatase inhibitors sodium fluoride (1 mM) and sodium orthovanadate (2 mM).
- 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



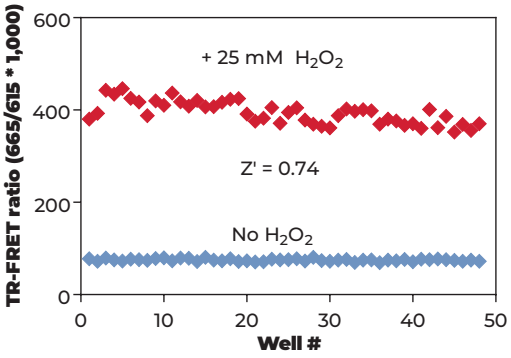
A431 cells (40,000 cells/well; in triplicate) were incubated with serial dilutions of H_2O_2 for 10 min at RT. Data show that treatment of A431 cells with H_2O_2 stimulates phosphorylation of SRC at Y419.

INHIBITION OF PHOSPHO-SRC (Y419) IN A431 CELLS



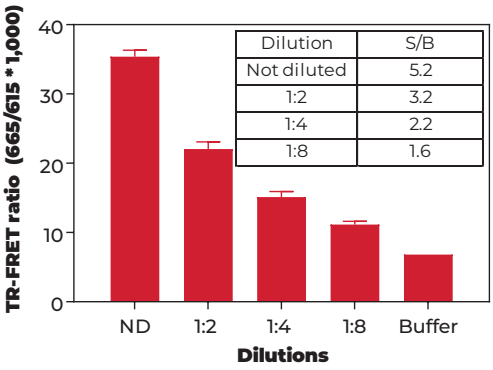
A431 cells (40,000 cells/well; in triplicate) were incubated with serial dilutions of the inhibitor Bosutinib for 10 min at RT. Cells were then stimulated with 20 mM of H_2O_2 for 10 min at RT. Data show that treatment of A431 cells with Bosutinib inhibits phosphorylation of SRC at Y419 by H_2O_2.

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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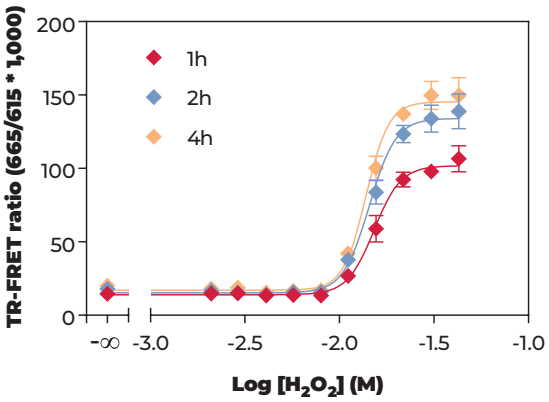
STIMULATION OF PHOSPHO-SRC (Y419) IN A549 CELLS



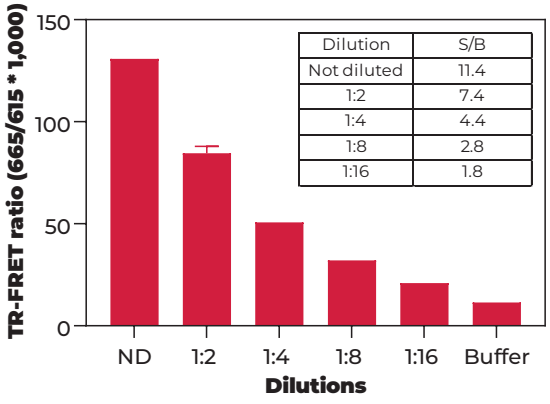
A549 cells (50,000 cells/well; in triplicate) were cultured overnight in a 96-well tissue culture plate. Cells were then incubated with serial dilutions of H_2O_2 for 10 min at RT. Data show that treatment of A549 cells with H_2O_2 stimulates phosphorylation of SRC at Y419.

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

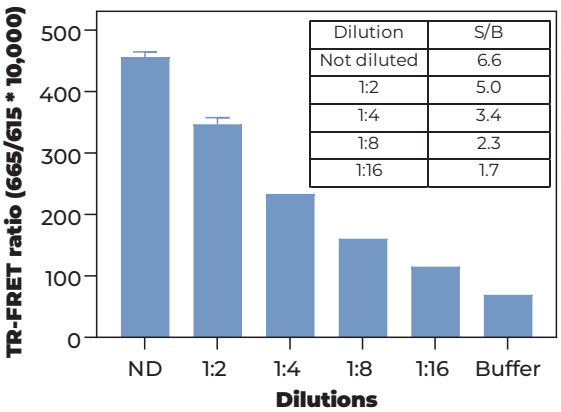
As part of assay validation, the THUNDER™ Phospho-SRC (Y419) Assay Kit was benchmarked against a competitive TR-FRET assay technology (Company B). Specifically, the 2 different assays were compared side-by-side for their capacity to detect phospho-SRC (Y419) stimulation upon A549 cell treatment with 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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