TECHNICAL DATA SHEET

THUNDER Phospho SMAD3 (S423/S425) + Total SMAD3 TR-FRET Cell Signaling Assay Kit

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CATALOG NUMBERS KIT-SMAD3PT-500 (500 tests)
400 points for phospho-SMAD3
and 100 points for total SMAD3

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-SMAD3 (S423/S425) and total SMAD3** 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 **SMAD3** phosphorylated at **Ser423** and **Ser425**, and another that recognizes **total** (both phosphorylated and unphosphorylated) **SMAD3**.

SPECIES REACTIVITY

Human and Mouse (Swiss-Prot Acc.: P84022; Entrez-Gene Id: 4088).

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

TR-FRET ASSAY PRINCIPLE

The **Phospho-SMAD3 (S423/S425) + Total SMAD3** 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-SMAD3 (S423/S425) + Total SMAD3** 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-SMAD3** or **total SMAD3**) 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-SMAD3 (S423/S425)** and **Total SMAD3** 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.

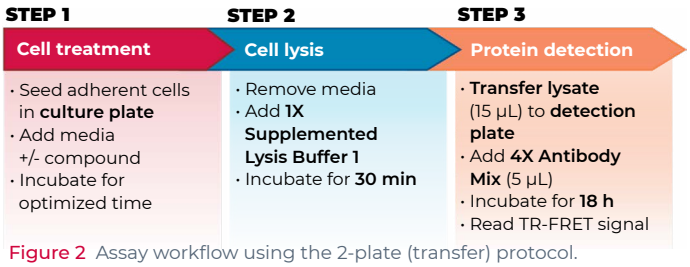


Figure 2 Assay workflow using the 2-plate (transfer) protocol.

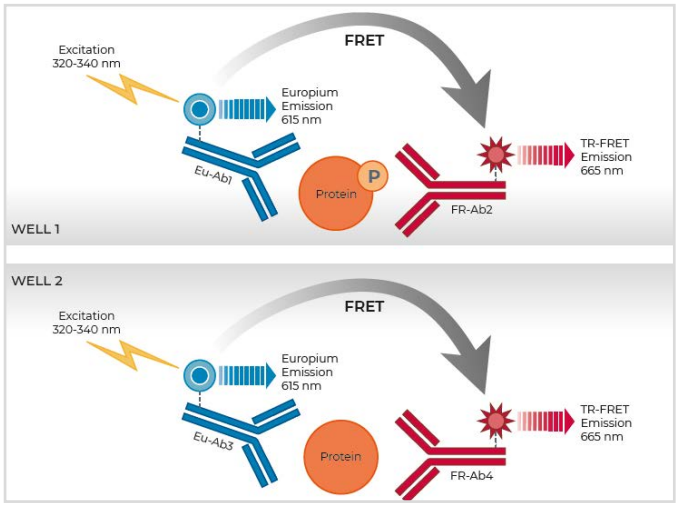


Figure 1 Schematic representation of the TR-FRET cell signaling assay principle.

KIT COMPONENTS

KIT COMPONENTS	500 points*
Eu-labeled phospho-SMAD3 (S423/S425) antibody (Eu-Ab1)	20 µL
Acceptor-labeled phospho-SMAD3 (S423/S425) antibody (FR-Ab2)	80 µL
Eu-labeled total-SMAD3 antibody (Eu-Ab3)	5 µL
Acceptor-labeled total-SMAD3 antibody (FR-Ab4)	20 µL
Lysis Buffer 1 (5X)	5 mL
Detection Buffer (10X)	250 µL
Positive control cell lysate	200 µL
Phosphatase Inhibitor Cocktail (100X)	250 µL

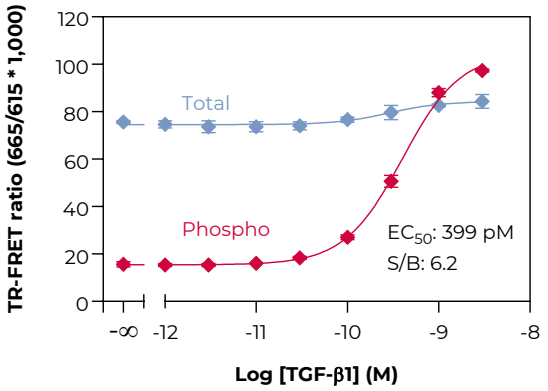
* The number of assay points is based on an assay volume of 20 µL in half-area 96-well or low-volume 384-well assay plates using the kit components at the recommended concentrations (refer to the User Manual).

VALIDATION DATA

This assay kit has been validated for the relative quantification of phospho SMAD3 (S423/S425) and total SMAD3 in A549 cell lysates using the 2-plate assay protocol.

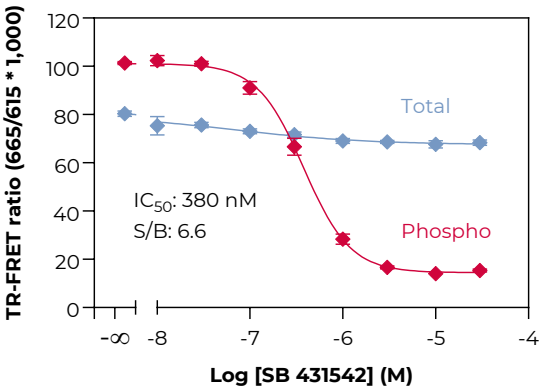
- Adherent cells were cultured overnight in a 96-well tissue culture plate (RPMI +10% FBS).
- Following cell treatment, the media was removed and cells were lysed with the 1X **Lysis Buffer 1** (50 µL) supplemented with the 100X Phosphatase Inhibitor Cocktail diluted at 1X.
- Following a **30-min** incubation at room temperature (RT) on an orbital shaker (400 rpm), lysates (15 µL) were 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 SMAD3 (S423/S425) or Eu-Ab3 and FR-Ab4 (5 µL) for detection of total SMAD3.
- 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).

STIMULATION OF PHOSPHO-SMAD3 (S423/S425) IN A549 CELLS



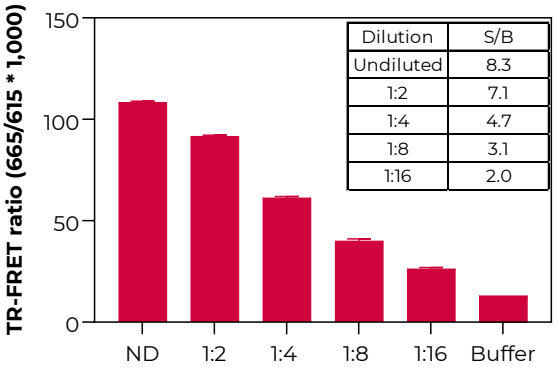
A549 cells (75,000 cells/well; in triplicate) were incubated with serial dilutions of TGF-β1 for 15 min at 37°C. Data show that treatment of A549 cells with TGF-β1 stimulates phosphorylation of SMAD3 at S423/S425, but does not have an effect on the levels of total SMAD3.

INHIBITION OF PHOSPHO-SMAD3 (S423/S425) IN A549 CELLS



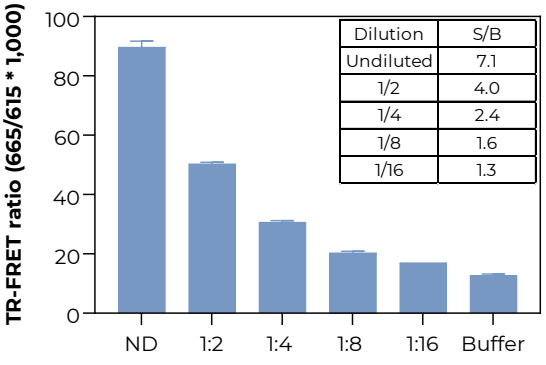
A549 cells (75,000 cells/well; in triplicate) were incubated with serial dilutions of the inhibitor SB 431542 for 15 min at 37°C. Cells were then stimulated with 0.5 nM of TGF-β1 for 15 min at 37°C. Data show that treatment of A549 cells with SB 431542 inhibits phosphorylation of SMAD3 at S423/S425 by TGF-β1 but does not have an effect on the levels of total SMAD3.

A549 CONTROL LYSATE TITRATION (QC TEST) PHOSPHO-SMAD3 (S423/S425)



Dilution	S/B
Undiluted	8.3
1:2	7.1
1:4	4.7
1:8	3.1
1:16	2.0

A549 CONTROL LYSATE TITRATION (QC TEST) TOTAL SMAD3



Dilution	S/B
Undiluted	7.1
1/2	4.0
1/4	2.4
1/8	1.6
1/16	1.3

Quality Control: the total SMAD3 assay kit is routinely tested against TGF-β1 treated A549 lysates. A549 cells were cultured in a T175 flask to 85% confluence and stimulated with 3 nM of TGF-β1 for 15 min at 37°C. Following cell lysis using 4 mL of 1X Lysis Buffer 1, lysates were serially diluted with 1X Lysis Buffer 1 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.