TECHNICAL DATA SHEET

THUNDER™ High Performance Phospho-STAT1 (Y701) TR-FRET Cell Signaling Assay Kit

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CATALOG NUMBERS

KIT-HP-STAT1P-100 (100 tests)
KIT-HP-STAT1P-500 (500 tests)
KIT-HP-STAT1P-2500 (2500 tests)
KIT-HP-STAT1P-5000 (5000 tests)
KIT-HP-STAT1P-10000 (10000 tests)

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

Visit Product Page

PRODUCT DESCRIPTION

This High Performance assay kit measures intracellular levels of **phospho-STAT1 (Y701)** 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 **STAT1** phosphorylated at **Tyr705** and another that recognizes an invariant epitope of **STAT1**.

SPECIES REACTIVITY

Human, mouse (Swiss-Prot Acc.: P42224; Entrez-Gene Id: 6772).

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

TR-FRET ASSAY PRINCIPLE

The **High Performance Phospho-STAT1 (Y701)** 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-STAT1 (Y701)** 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-STAT1 (Y701)** in the cell lysate. Data can be expressed as either the signal at 665 nm or the 665 nm/615 nm ratio.

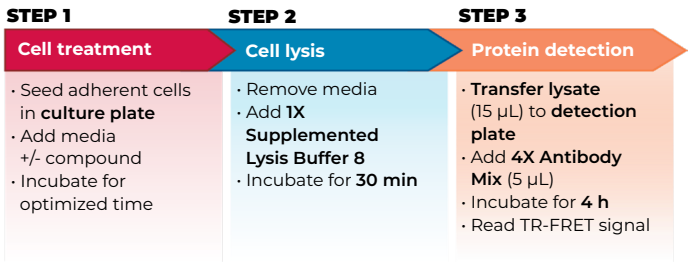


Figure 2 Assay workflow using the 2-plate (transfer) protocol.

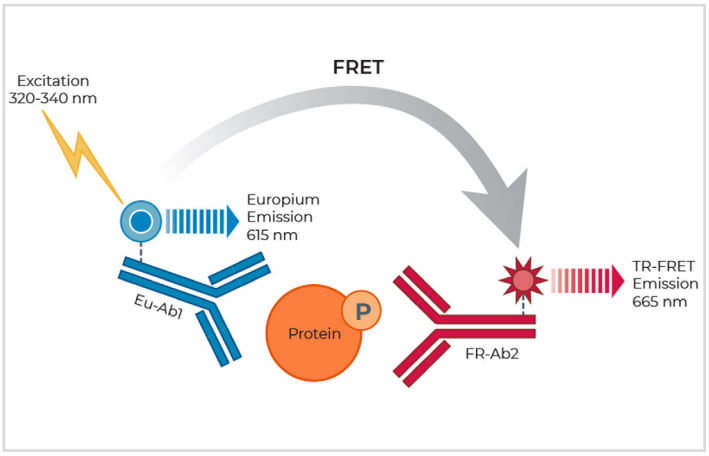


Figure 1 Schematic representation of the TR-FRET cell signaling assay principle.

KIT COMPONENTS

KIT COMPONENTS	100 points*	500 points*
Eu-labeled HP-phospho-STAT1 (Y701) antibody (Eu-Ab1)	5 µL	25 µL
Acceptor-labeled HP-phospho-STAT1 (Y701) antibody (FR-Ab2)	20 µL	100 µL
Lysis Buffer 8 (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

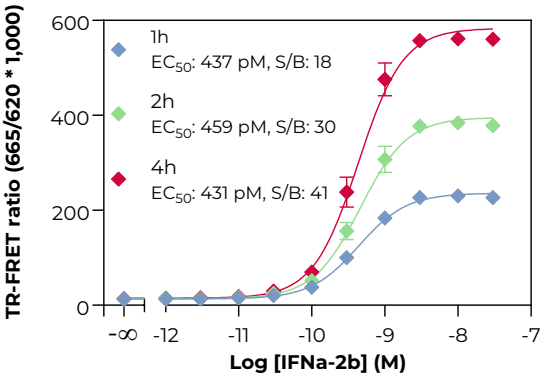
* The number of assay points is based on an assay volume of 20 µL in half-area 96-well or low-volume 384-well assay plates using the kit components at the recommended concentrations (refer to the User Manual).

VALIDATION DATA

This assay kit has been validated for the relative quantification of phospho-STAT1 (Y701) in HeLa cell lysates using the 2-plate assay protocol. This kit has also been validated for the detection of phospho-STAT1 (Y701) in the murine cell line NIH/3T3.

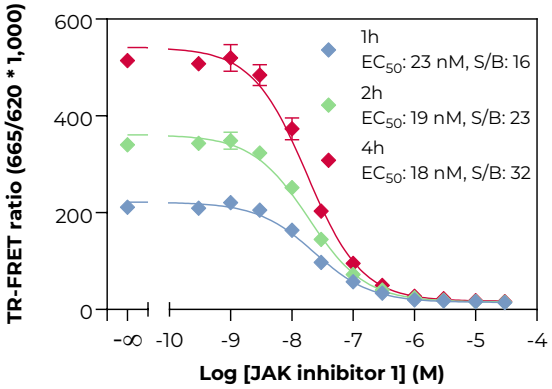
- Adherent cells were cultured overnight in a 96-well tissue culture plate (DMEM + 10% FBS).
- Following cell treatment, the media was removed and cells were lysed with the 1X **Lysis Buffer 8** (50 µL) supplemented with the 100X Phosphatase Inhibitor Cocktail diluted at 1X.
- Following a **30-min** 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-STAT1 (Y701).
- The plate was incubated at RT for **4 hours** (unless otherwise indicated) and the TR-FRET signal was recorded at 665 and 620 nm (PHERASTAR® FSX; laser excitation).

STIMULATION OF PHOSPHO-STAT1 (Y701) IN HELA CELLS



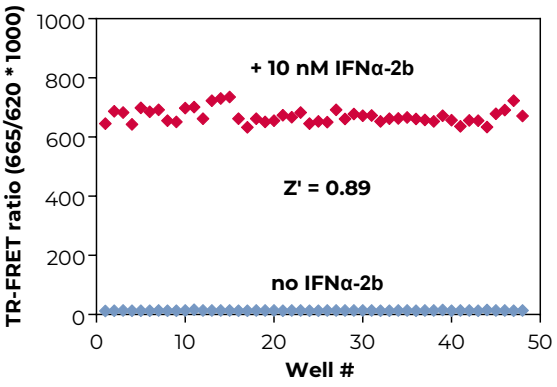
HeLa cells (100,000 cells/well; in triplicate) were incubated with serial dilutions of IFNα-2b for 20 min at RT. Data show that treatment of HeLa cells with IFNα-2b stimulates phosphorylation of STAT1 at Y701.

INHIBITION OF PHOSPHO-STAT1 (Y701) IN HELA CELLS



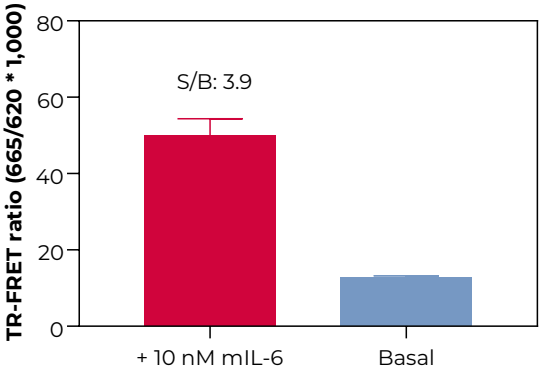
HeLa cells (100,000 cells/well; in triplicate) were incubated with serial dilutions of JAK Innibitor 1 for 30 min at 37°C. Cells were then stimulated with 1.9 nM of IFNα-2b for 20 min at RT. Data show that treatment of HeLa cells with JAK Innibitor 1 inhibits phosphorylation of STAT1 at Y701 by IFNα-2b.

Z'-FACTOR DETERMINATION IN HELA CELLS



HeLa cells (100,000 cells/well) were incubated without or with 10 nM of IFNα-2b for 60 min at RT. The Z' factor value was determined after a 4h incubation time, using a total of 48 wells for each treatment group. The Z'-factor value of 0.89 indicates that the assay is robust and suitable for HTS.

STIMULATION OF PHOSPHO-STAT1 (Y701) IN NIH/3T3 CELLS



NIH/3T3 cells (50,000 cells/well; in triplicate) were incubated with 10 nM of mIL-6 for 20 min at RT. Data show that treatment of NIH/3T3 cells with mIL-6 stimulates phosphorylation of STAT1 at Y701.

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