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# Produktinformation

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- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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## Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

## Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand


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TECHNICAL DATA SHEET

# THUNDER™ Phospho-STAT6 (Y641) + Total STAT6
## TR-FRET Cell Signaling Assay Kit

bioauxilium
BETTER TOOLS. REAL DISCOVERIES.

**CATALOG NUMBERS** KIT-STAT6PT-500
400 points for phospho-STAT6 and 100 points for total STAT6

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

## PRODUCT DESCRIPTION

This assay kit measures intracellular levels of **Phospho-STAT6 (Y641)** and **total STAT6** 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 **STAT6** phosphorylated at **Tyr641** and another that recognizes **total** (both phosphorylated and unphospho-rylated) **STAT6.**

## SPECIES REACTIVITY

Human (Swiss-Prot Acc.: P42226; Entrez-Gene Id: 6778).

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

## TR-FRET ASSAY PRINCIPLE

The **Phospho-STAT6 (Y641) + Total STAT6** assay kit is a homo-geneous 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-STAT6 (Y641)** and **Total STAT6** 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-STAT6** or **total STAT6**) 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-STAT6 (Y641)** and **Total STAT6** in the cell lysate. Data can be expressed as either the signal at 665 nm or the 665 nm/615 nm ratio.

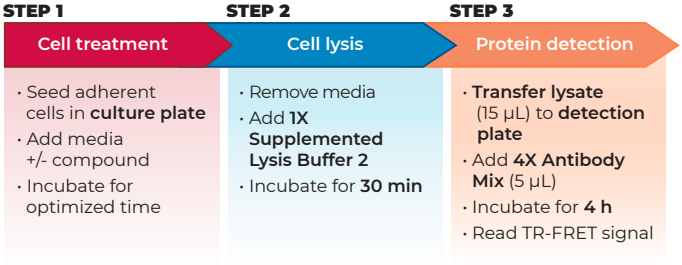



Figure 2 Assay workflow using the 2-plate (transfer) protocol.

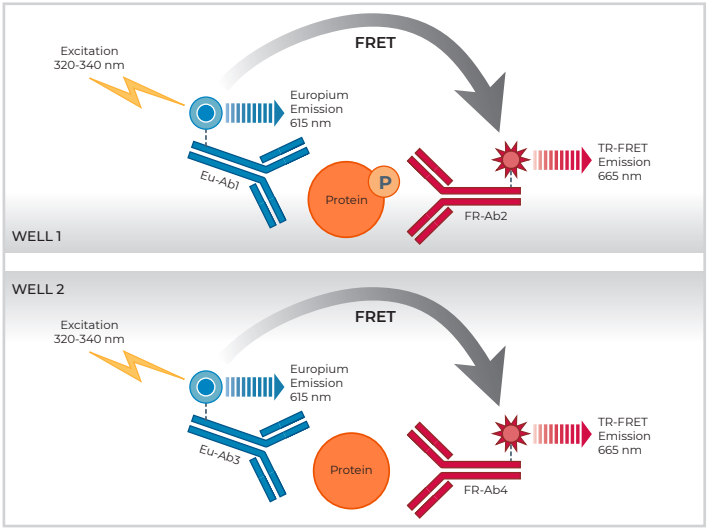



Fig. 1 Schematic representation of the TR-FRET cell signaling assay principle.

## KIT COMPONENTS

| KIT COMPONENTS | 500 points* |
|---|---|
| Eu-labeled Phospho-STAT6 (Y641) antibody (Eu-Ab1) | 20 µL |
| Acceptor-labeled Phospho-STAT6 (Y641) antibody (FR-Ab2) | 80 µL |
| Eu-labeled total-STAT6 antibody (Eu-Ab3) | 5 µL |
| Acceptor-labeled total-STAT6 antibody (FR-Ab4) | 20 µL |
| Lysis Buffer 2 (5X) | 5 mL |
| Detection Buffer (10X) | 250 µL |
| Positive control cell lysate | 500 µL |

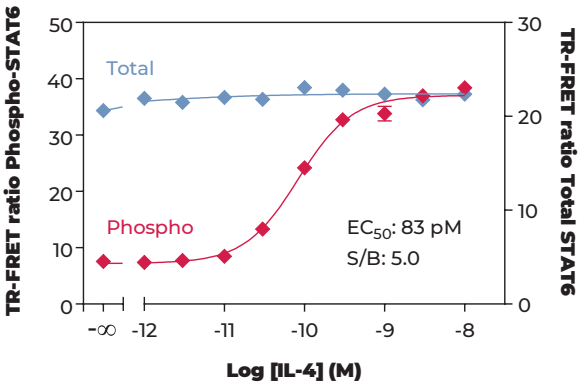
* The number of assay points is based on an assay volume of 20 µL in half-area 96-well or low-volume 384-well assay plates using the kit components at the recommended concentrations (refer to the User Manual).

## VALIDATION DATA

This assay kit has been validated for the relative quantification of Phospho-STAT6 (Y641) and total STAT6 in HeLa cell lysates using the 2 plate assay protocol.

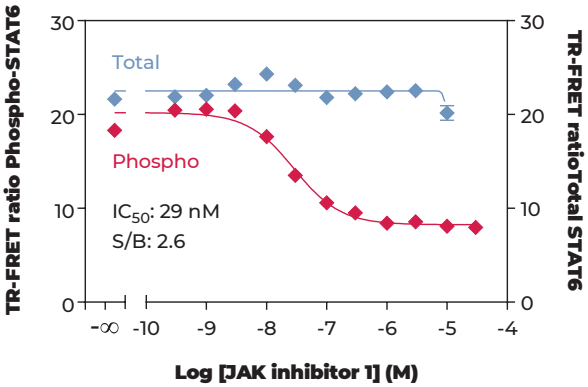
- Adherent cells were cultured overnight in a 96-well tissue culture plate (DMEM +10% FBS).
- Following cell treatment, the media was removed and cells were lysed with the 1X **Lysis Buffer 2** (50 µL) supplemented with the phosphatase inhibitors sodium fluoride (1 mM) and sodium orthovanadate (2 mM).
- 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 to separate wells of either the labeled antibodies Eu-Ab1 and FR-Ab2 (5 µL) for detection of phospho-STAT6 (Y641) or Eu-Ab3 and FR-Ab4 (5 µL) for detection of total STAT6.
- The plate was incubated at RT for **4 hours** and the TR-FRET signal was recorded at 665 and 615 nm (EnVision®; lamp excitation).

### STIMULATION OF PHOSPHO-STAT6 (Y641) IN HELA CELLS



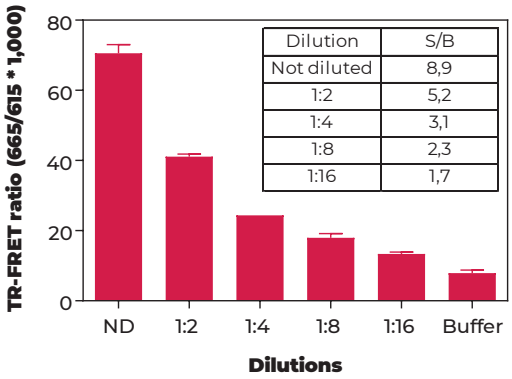

HeLa cells (40,000 cells/well; in triplicate) were incubated with serial dilutions of IL-4 for 20 min at RT. Data show that treatment of HeLa cells with IL-4 stimulates phosphorylation of STAT6 at Y641, but does not affect the levels of total STAT6.

### INHIBITION OF PHOSPHO-STAT6 (Y641) IN HELA CELLS



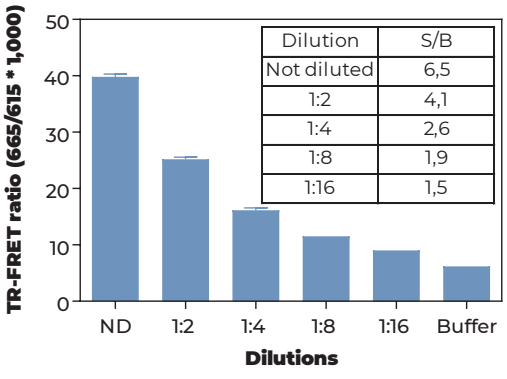

HeLa cells (40,000 cells/well; in triplicate) were incubated with serial dilutions of the inhibitor JAK Inhibitor 1 for 30 min at RT. Cells were then stimulated with 500 pM of IL-4 for 20 min at RT. Data show that treatment of HeLa cells with JAK Inhibitor 1 inhibits phosphorylation of STAT6 at Y641 by IL-4, but does not affect the levels of total STAT6.

### HELA CONTROL LYSATE TITRATION (QC TEST) PHOSPHO-STAT6 (Y641)




| Dilution | S/B |
|---|---|
| Not diluted | 8,9 |
| 1:2 | 5,2 |
| 1:4 | 3,1 |
| 1:8 | 2,3 |
| 1:16 | 1,7 |

### HELA CONTROL LYSATE TITRATION (QC TEST) TOTAL STAT6




| Dilution | S/B |
|---|---|
| Not diluted | 6,5 |
| 1:2 | 4,1 |
| 1:4 | 2,6 |
| 1:8 | 1,9 |
| 1:16 | 1,5 |

Quality Control: the Phospho-STAT6 (Y641) + Total STAT6 assay kit is routinely tested against IL-4-treated HeLa lysates. HeLa cells were cultured in a T175 flask to 60% confluence and stimulated with 1 nM of IL-4 for 20 min at RT. Following cell lysis using 3 mL of 1X Lysis Buffer 2, lysates were serially diluted with 1X Lysis Buffer 2 and tested in triplicate and in separate wells for phospho-STAT6 (Y641) and total STAT6. 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.**

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