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TECHNICAL DATA SHEET

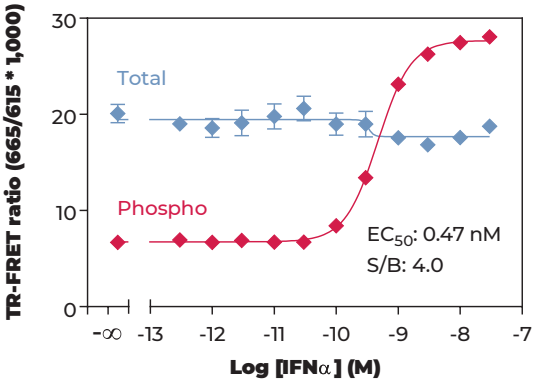
Phospho-STAT3 (Y705) + Total STAT3

VALIDATION DATA

This assay kit has been validated for the relative quantification of phospho-STAT3 (Y705) and total STAT3 in HeLa cell lysates using the 2 plate assay protocol.

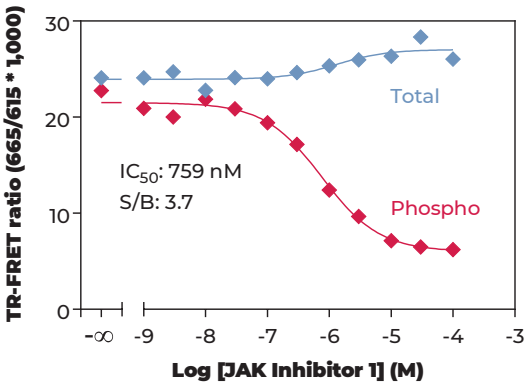
- Adherent cells were cultured overnight in a 96-well tissue culture plate (DMEM +10% FBS).
- Following cell treatment, the media was removed and cells were lysed with the 1X **Lysis Buffer 2** (50 µL) supplemented with the phosphatase inhibitors sodium fluoride (1 mM) and sodium orthovanadate (2 mM).
- 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-STAT3 (Y705) or Eu-Ab3 and FR-Ab4 (5 µL) for detection of total STAT3.
- 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-STAT3 (Y705) IN HELA CELLS



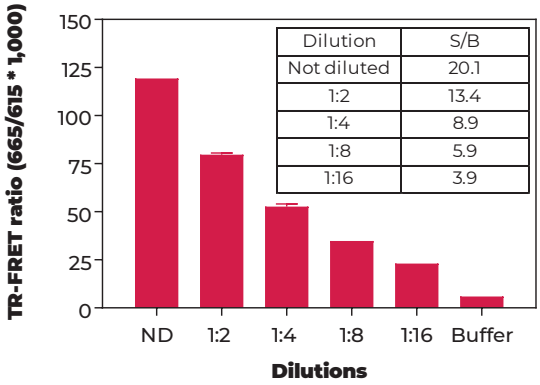
HeLa cells (40,000 cells/well; in triplicate) were incubated with serial dilutions of IFNα for 30 min at RT. Data show that treatment of HeLa cells with IFNα stimulates phosphorylation of STAT3 (Y705), but does not affect the levels of total STAT3.

INHIBITION OF PHOSPHO-STAT3 (Y705) IN HELA CELLS



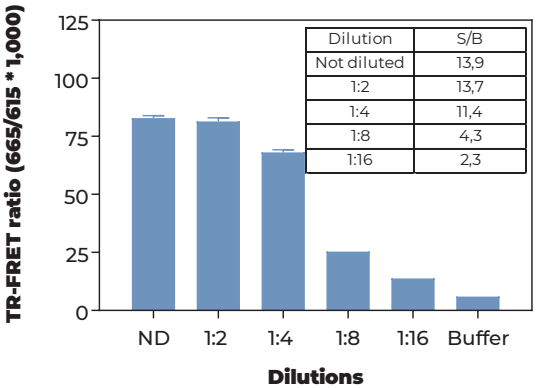
HeLa cells (40,000 cells/well; in triplicate) were incubated with serial dilutions of JAK Innibitor 1 for 30 min at RT. Cells were then stimulated with 1.5 nM of IFNα for 30 min at RT. Data show that treatment of HeLa cells with JAK Innibitor 1 inhibits phosphorylation of STAT3 (Y705) by IFNα, but does not affect the levels of total STAT3.

HELA CONTROL LYSATE TITRATION (QC TEST) PHOSPHO-STAT3 (Y705)



Dilution	S/B
Not diluted	20.1
1:2	13.4
1:4	8.9
1:8	5.9
1:16	3.9

HELA CONTROL LYSATE TITRATION (QC TEST) TOTAL STAT3



Dilution	S/B
Not diluted	13,9
1:2	13,7
1:4	11,4
1:8	4,3
1:16	2,3

Quality Control: the Phospho-STAT3 (Y705) + Total STAT3 assay kit is routinely tested against IFNα-treated HeLa lysates. HeLa cells were cultured in a T175 flask to 90% confluence and stimulated with 3 nM of IFNα for 30 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 and in separate wells for phospho-STAT3 (Y705) and total STAT3. Data show a linear relationship between lysate dilutions and TR-FRET ratio values. Note that due to the very high sensitivity of the Total STAT3 kit, lysates from the T175 flask required at least a 1:4 pre-dilution in order to be within the dynamic assay range.

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

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