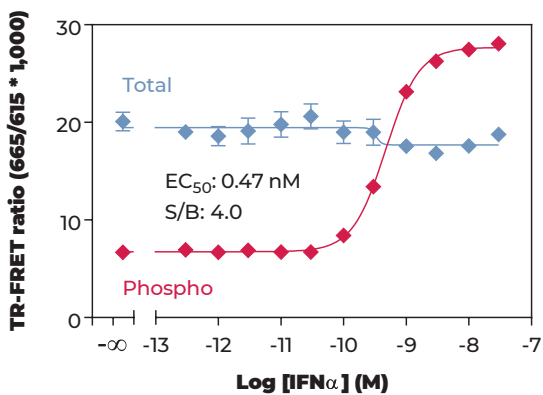


VALIDATION DATA

This assay kit has been validated for the relative quantification of total STAT3 in HeLa cell lysates using the 2 plate assay protocol.

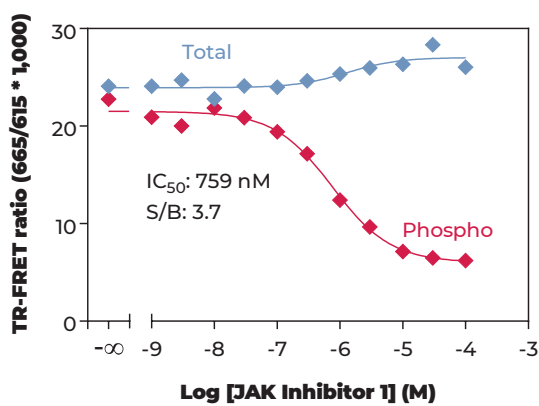
- Adherent cells were cultured overnight in a 96-well tissue culture plate (DMEM +10% FBS).
- Following cell treatment, the media was removed and cells were lysed with the 1X **Lysis Buffer 2** (50 μ L) supplemented with the phosphatase inhibitors sodium fluoride (1 mM) and sodium orthovanadate (2 mM).
- Following a **30-min** incubation at room temperature (RT) on an orbital shaker (400 rpm), lysates (15 μ L) were then transferred to a 384-well assay plate followed by addition of the labeled antibodies Eu-Ab1 and FR-Ab2 (5 μ L) 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



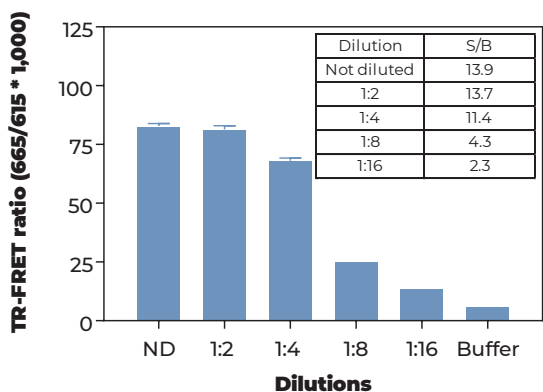
HeLa cells (40,000 cells/well; in triplicate) were incubated with serial dilutions of IFN α for 30 min at RT. Data show that treatment of HeLa cells with IFN α stimulates phosphorylation of STAT3 at Y705, but does not affect the levels of total STAT3.

INHIBITION OF PHOSPHO-STAT3 (Y705) IN HELA CELLS



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

HELA CONTROL LYSATE TITRATION (QC TEST)



Quality Control: the Total STAT3 assay kit is routinely tested against IFN α treated HeLa lysates. HeLa cells were cultured in a T175 flask to 90% confluence and stimulated with 3 nM of IFN α 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. 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.

