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TECHNICAL DATA SHEET

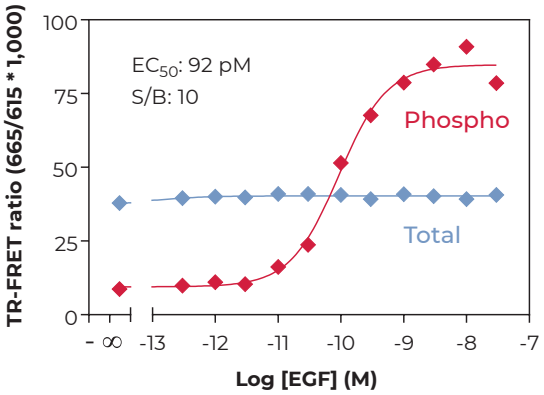
Total ERK1/2

VALIDATION DATA

This assay kit has been validated for the relative quantification of total ERK1/2 in HEK293 cell lysates using the 2-plate assay protocol.

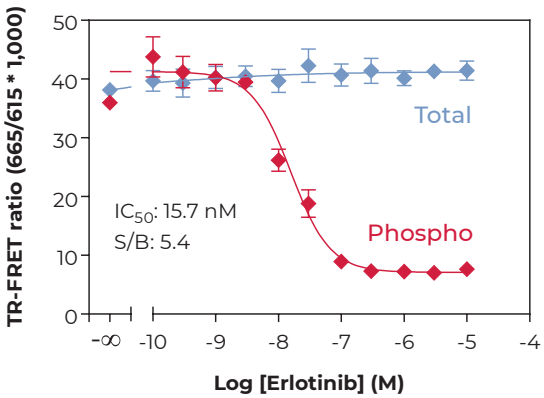
- Adherent cells were cultured overnight in a 96-well tissue culture plate coated with poly-L-lysine (EMEM +10% FBS).
- Following cell treatment, the media was removed and cells were lysed with the 1X **Lysis Buffer 1** (50 µL) supplemented with the phosphatase inhibitors sodium fluoride (1 mM) and sodium 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 of the labeled antibodies Eu-Ab1 and FR-Ab2 (5 µL) for detection of total ERK1/2.
- 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-ERK1/2 (T202/Y204) IN HEK293 CELLS



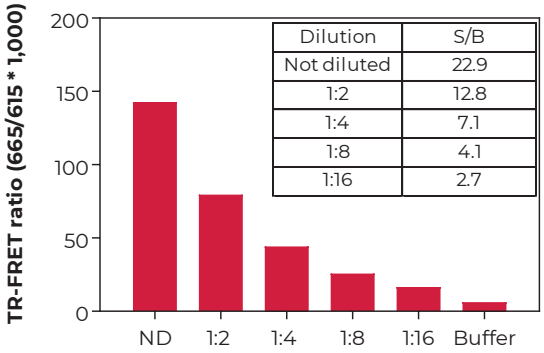
HEK293 cells (50,000 cells/well; in triplicate) were incubated with serial dilutions of EGF for 10 min at RT. Data show that treatment of HEK293 cells with EGF stimulates phosphorylation of ERK1/2 at T202/Y204, but does not affect the levels of total ERK1/2.

INHIBITION OF PHOSPHO-ERK1/2 (T202/Y204) IN HEK293 CELLS



HEK293 cells (50,000 cells/well, in triplicate) were incubated with serial dilutions of the inhibitor Erlotinib for 15 min at RT. Cells were then stimulated with 0.5 nM of EGF for 10 min at RT. Data show that treatment of HEK293 cells with Erlotinib inhibits phosphorylation of ERK1/2 at T202/Y204 by EGF, but does not affect the levels of total ERK1/2.

HEK293 CONTROL LYSATE TITRATION (QC TEST)



Dilution	S/B
Not diluted	22.9
1:2	12.8
1:4	7.1
1:8	4.1
1:16	2.7

Quality Control: the Total ERK1/2 assay kit is routinely tested against EGF-treated HEK293 lysates. HEK293 cells were cultured in a T175 flask to 90% confluence and stimulated with 30 nM of EGF for 10 min at RT. Following cell lysis using 5 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.

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