**TECHNICAL DATA SHEET**

# THUNDER™
# Extreme Phospho-ERK1/2 (T202/Y204) TR-FRET Cell Signaling Assay Kit

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BETTER TOOLS. REAL DISCOVERIES.

**CATALOG NUMBERS**

**KIT-XTM-ERKP-100** (100 tests)
**KIT-XTM-ERKP-500** (500 tests)
**KIT-XTM-ERKP-2500** (2500 tests)
**KIT-XTM-ERKP-5000** (5000 tests)
**KIT-XTM-ERKP-10000** (10000 tests)

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

**Visit product page**

## PRODUCT DESCRIPTION

This high-sensitivity assay kit measures low intracellular levels of **phospho-ERK1/2 (T202/Y204)** 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, and is suitable for measuring the inhibition of basal phospho-ERK1/2 levels.

## SPECIFICITY

This assay kit contains two specific and selective antibodies, one that recognizes **ERK1/2** phosphorylated at **Thr202** and **Tyr204** and another that recognizes an invariant epitope of **ERK 1/2**.

## SPECIES REACTIVITY

Human, Mouse (Swiss-Prot Acc.: P27361, P28482; Entrez-Gene Id: 5595, 5594).

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

## TR-FRET ASSAY PRINCIPLE

The **Extreme Phospho-ERK1/2 (T202/Y204)** 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-ERK1/2 (T202/Y204)** 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-ERK1/2 (T202/Y204)** in the cell lysate. Data can be expressed as either the signal at 665 nm or the 665 nm/615 nm ratio.

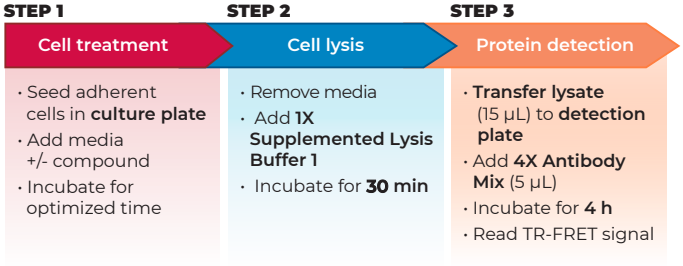


Figure 2 Assay workflow using the 2-plate (transfer) protocol.

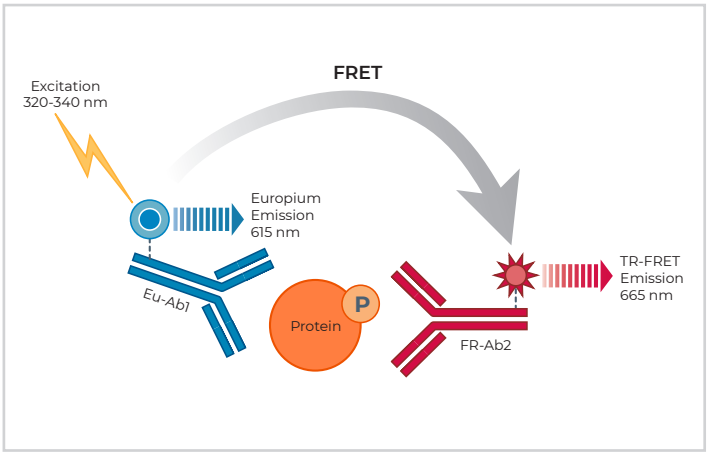


Figure 1 Schematic representation of the TR-FRET cell signaling assay principle.

## KIT COMPONENTS

| | 100 points* | 500 points* |
|---|---|---|
| Eu-labeled phospho-XTMERK1/2 (T202/Y204) antibody (Eu-Ab1) | 5 µL | 25 µL |
| Acceptor-labeled phospho-XTMERK1/2 (T202/Y204) antibody (FR-Ab2) | 20 µL | 100 µL |
| Lysis Buffer 1 (5X) | 1 mL | 5 mL |
| Detection Buffer (10X) | 50 µL | 250 µL |
| Positive control cell lysate | 100 µL | 200 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).

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# TECHNICAL DATA SHEET

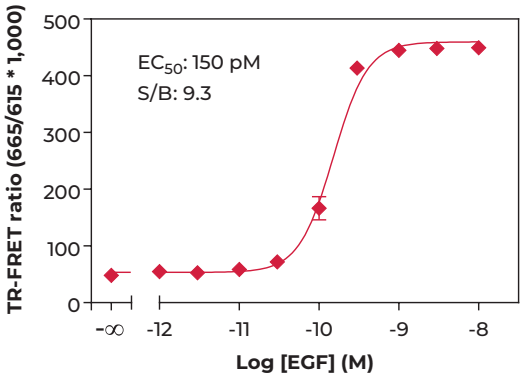
Extreme Phospho-ERK1/2 (T202/Y204)

## VALIDATION DATA

This assay kit has been validated for the relative quantification of phospho-ERK1/2 (T202/Y204) in HEK293, H358, MCF7 and B lymphocyte lysates using the 2-plate assay protocol.

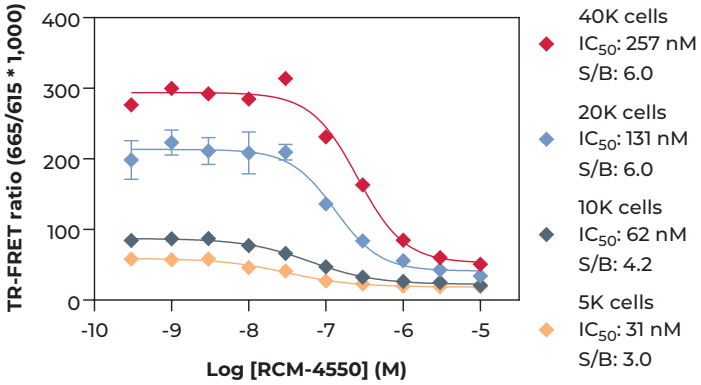
- Adherent cells were cultured overnight in a 96-well tissue culture plate (HEK293: coated with poly-L-lysine). Media was EMEM +10% FBS (HEK293 and MCF7 cells), RPMI +10% FBS (H358) or RPMI +15% FBS (B lymphocytes).
- Following cell treatment, the media was removed (adherent cells) and cells were lysed with either 1X Lysis Buffer 1 (50 µL) or 5X Lysis Buffer 1 (10 µL; suspension cells) supplemented with the 100X Phosphatase Inhibitor Cocktail diluted at 1X or 5X, respectively.
- 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-ERK1/2 (T202/Y204).
- 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



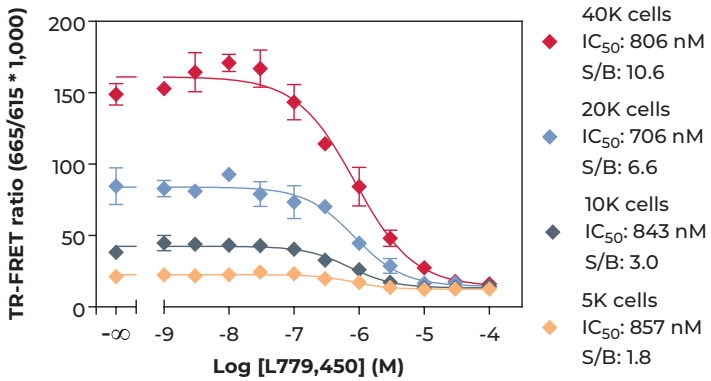

HEK293 cells (50,000 cells/well; in triplicate) were incubated with serial dilutions of EGF for 10 min at RT. Data show that treatment of HEK293 cells with EGF stimulates phosphorylation of ERK1/2 at T202/Y204.

### INHIBITION OF BASAL PHOSPHO-ERK1/2 (T202/Y204) IN H358 CELLS



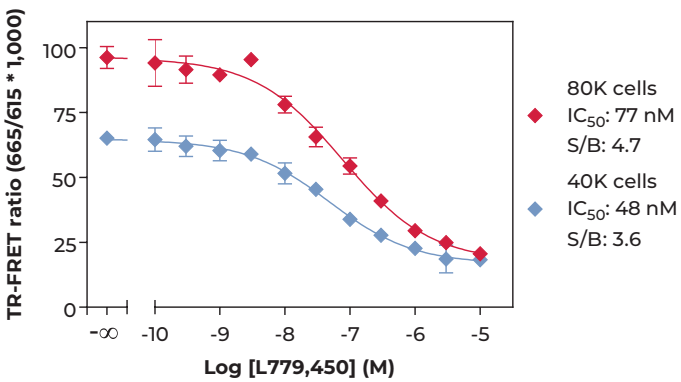

H358 cells (5K, 10K, 20K or 40K cells/well, in triplicate) were cultured overnight and then serum starved for 18 hours. Cells were then incubated with serial dilutions of the inhibitor RMC-4550 for 60 min at 37°C. Data show that treatment of H358 cells with RMC-4550 inhibits basal phosphorylation of ERK1/2 at T202/Y204.

### INHIBITION OF BASAL PHOSPHO-ERK1/2 (T202/Y204) IN MCF7 CELLS




MCF7 cells (5K, 10K, 20K or 40K cells/well, in triplicate) were cultured overnight. Cells were then incubated with serial dilutions of the inhibitor L779,450 for 30 min at 37°C. Data show that treatment of MCF7 cells with L779,450 inhibits basal phosphorylation of ERK1/2 at T202/Y204.

### INHIBITION OF BASAL PHOSPHO-ERK1/2 (T202/Y204) IN B LYMPHOCYTES




B lymphocyte cells (40K or 80K cells/well, in triplicate) resuspended in RPMI without serum were incubated with serial dilutions of the inhibitor L779,450 for 30 min at RT. Data show that treatment of B lymphocyte cells with L779,450 inhibits basal phosphorylation of ERK1/2 at T202/Y204.

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

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