

TECHNICAL DATA SHEET



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BETTER TOOLS. REAL DISCOVERIES.

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

CATALOG NUMBERS KIT-ERKP-100 (100 tests)
KIT-ERKP-500 (500 tests)

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

PRODUCT DESCRIPTION

This assay kit measures 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.

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 **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.

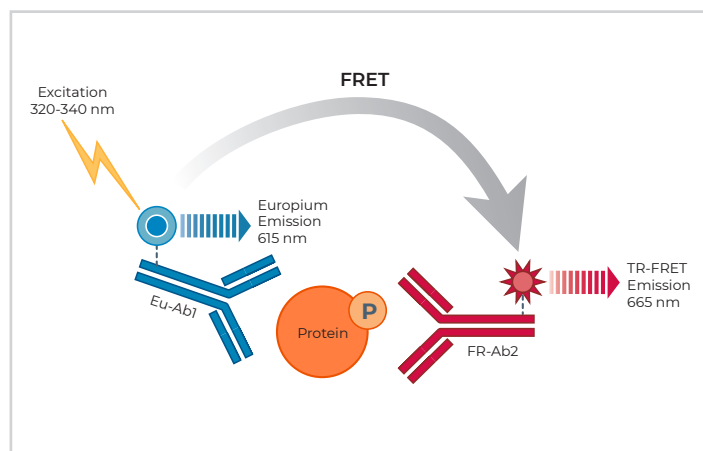


Figure 1 Schematic representation of the TR-FRET cell signaling assay principle.

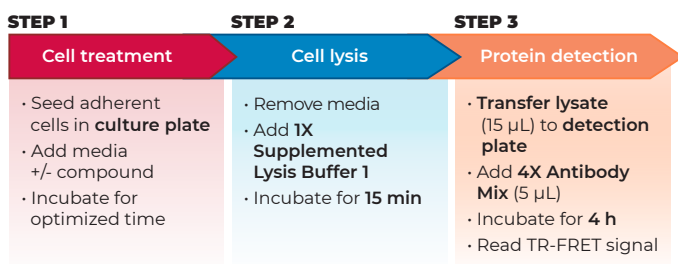


Figure 2 Assay workflow using the 2-plate (transfer) proto-

KIT COMPONENTS

	100 points*	500 points*
Eu-labeled phospho-ERK1/2 (T202/Y204) antibody (Eu-Ab1)	5 µL	25 µL
Acceptor-labeled phospho-ERK1/2 (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	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).

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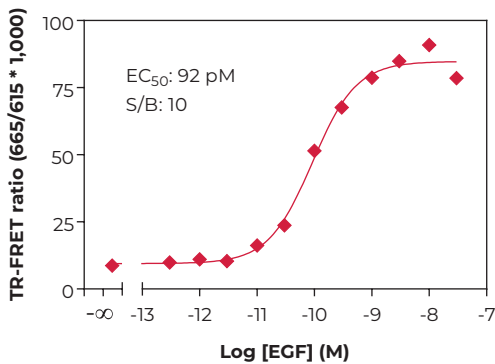
Phospho-ERK1/2 (T202/Y204)

VALIDATION DATA

This assay kit has been validated for the relative quantification of phospho-ERK1/2 (T202/Y204) in HEK293 cell lysates using the 2-plate assay protocol.

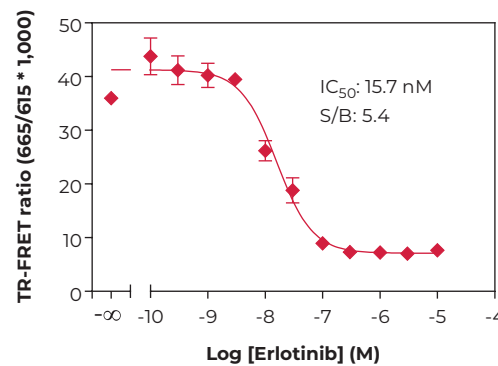
- Adherent cells were cultured overnight in a 96-well tissue culture plate coated with poly-L-lysine (EMEM +10% FBS).
- Following cell treatment, the media was removed and cells were lysed with the 1X **Lysis Buffer 1** (50 μ L) supplemented with the phosphatase inhibitors sodium fluoride (1 mM) and sodium orthovanadate (2 mM).
- Following a **15-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 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



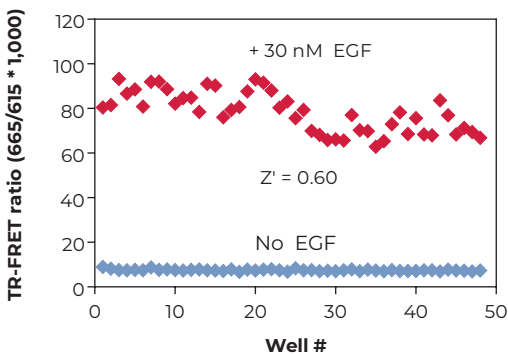
HEK293 cells (50,000 cells/well; in triplicate) were incubated with serial dilutions of EGF for 10 min at RT. Data show that treatment of HEK293 cells with EGF stimulates phosphorylation of ERK1/2 at T202/Y204.

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



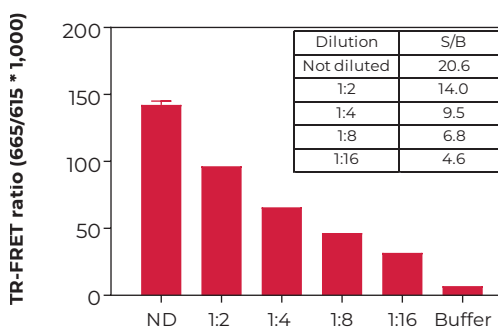
HEK293 cells (50,000 cells/well; in triplicate) were incubated with serial dilutions of the inhibitor Erlotinib for 15 min at RT. Cells were then stimulated with 0.5 nM of EGF for 10 min at RT. Data show that treatment of HEK293 cells with Erlotinib inhibits phosphorylation of ERK1/2 at T202/Y204 by EGF.

Z'-FACTOR DETERMINATION IN HEK293 CELLS



HEK293 cells (50,000 cells/well) were incubated without or with 30 nM of EGF for 10 min at RT. The Z' factor value was determined using a total of 48 wells for each treatment group. The Z'-factor value of 0.6 indicates that the assay is robust and suitable for HTS.

HEK293 CONTROL LYSATE TITRATION (QC TEST)



Quality Control: the Phospho-ERK1/2 (T202/Y204) 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.



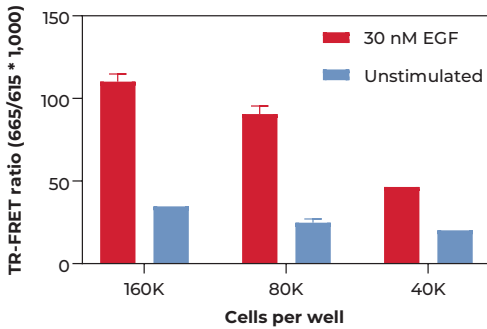
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TECHNICAL DATA SHEET

Phospho-ERK1/2 (T202/Y204)

ALL-IN-ONE-WELL PHOSPHO-ERK1/2 (T202/Y204) ASSAY



Cells/well	160K	80K	40K
S/B	3.2	3.6	2.3

HEK293 cells (at different cell densities; in triplicate) were plated in 8 μ L in a 384-well assay plate and immediately incubated with 4 μ L of EGF for 10 min at RT. Cells were then lysed with 3 μ L of 5X supplemented Lysis Buffer 1. Following a 15-min incubation at RT on an orbital shaker (400 rpm), 5 μ L of labeled antibodies Eu-Ab1 and FR-Ab2 were added directly to the lysate for detection of phospho-ERK1/2 (T202/Y204) in the same well, without a transfer step (1-plate assay protocol). Data show that stimulation of phospho-ERK1/2 at T202/Y204 can be detected using the 1-plate assay protocol.

2-PLATE ASSAY PROTOCOL USING HALF-AREA 96-WELL MICROPLATES

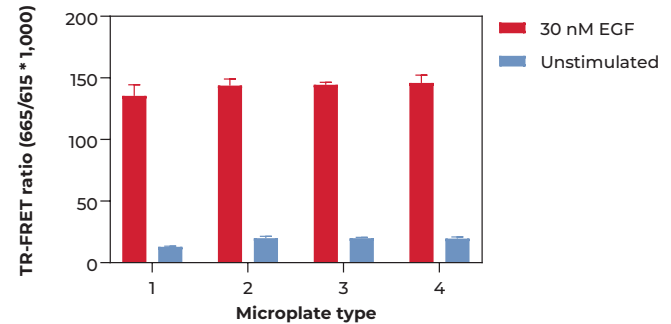


Plate	1	2	3	4
PerkinElmer 384-well LV		Greiner 96-well HA	PerkinElmer 96-well HA	Costar 96-well HA
S/B	10.4	7.2	7.2	7.4

HEK293 cells (50,000 cells/well; in triplicate) were incubated with 30 nM EGF for 10 min at RT. Following lysis, lysates (15 μ L) were transferred to either a low-volume (LV) 384-well assay plate (1: OptiPlate™, PerkinElmer) or different half-area (HA) 96-well assay plates (2: Greiner; 3: PerkinElmer; 4: Costar). This was followed by addition of the labeled antibodies Eu-Ab1 and FR-Ab2 (5 μ L). After 4 hours of incubation, plates were read on an EnVision® (PerkinElmer; lamp excitation). Data show that detection of phospho-ERK1/2 (T202/Y204) using the 2-plate transfer protocol can be conducted in different HA 96-well microplates.

2-PLATE ASSAY PROTOCOL USING HALF-AREA 96-WELL MICROPLATES SPARK® (TECAN)

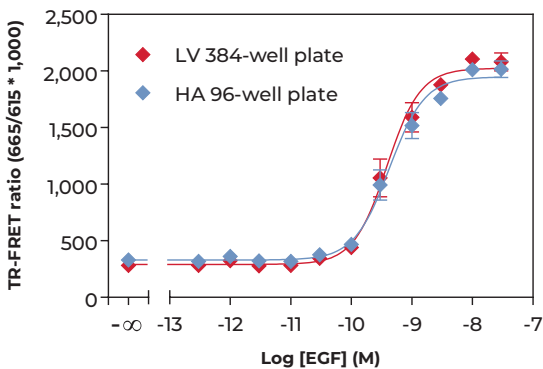


Plate	384-well	96-well
EC ₅₀ (nM)	0.39	0.42
S/B	7.4	6.1

HEK293 cells (50,000 cells/well; in triplicate) were incubated with serial dilutions of EGF for 10 min at RT. Following lysis, lysates (15 μ L) were transferred to either a low-volume (LV) 384-well assay plate (OptiPlate™, PerkinElmer) or a half-area (HA) 96-well assay plate (Greiner). This was followed by addition of the labeled antibodies Eu-Ab1 and FR-Ab2 (5 μ L). After 4 hours of incubation, plates were read on both an EnVision® (PerkinElmer) and a Spark® (TECAN) using lamp excitation. Data show that detection of phospho-ERK1/2 (T202/Y204) using the 2-plate transfer protocol can be conducted in both HA 96-well and LV 384-well plates with similar performance.

2-PLATE ASSAY PROTOCOL USING HALF-AREA 96-WELL MICROPLATES ENVISION® (PERKINELMER)

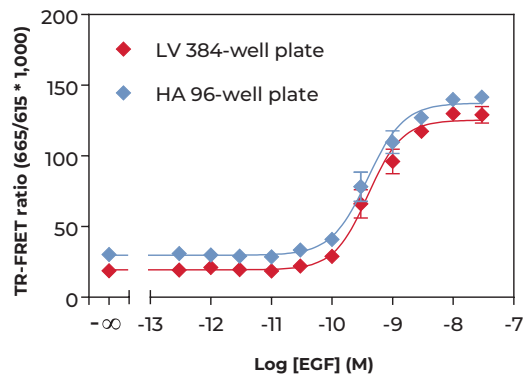


Plate	384-well	96-well
EC ₅₀ (nM)	0.39	0.38
S/B	6.9	4.7



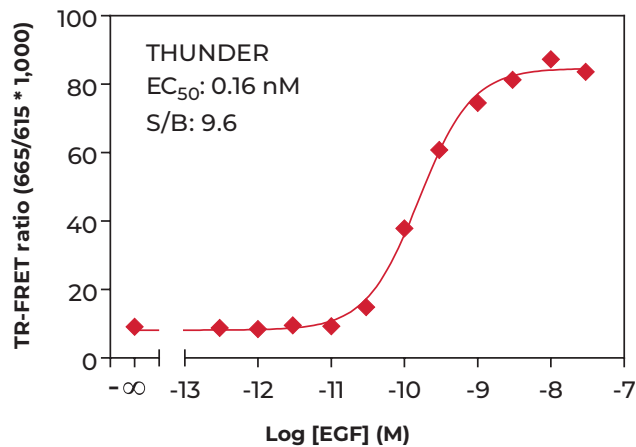
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KIT BENCHMARKING – COMPARISON TO OTHER TR-FRET TECHNOLOGIES

As part of assay validation, the THUNDER™ Phospho-ERK1/2 (T202/Y204) Assay Kit was benchmarked against a competitive TR-FRET assay technology (Company B). Specifically, the 2 different assays were compared side-by-side for their capacity to detect phospho-ERK1/2 (T202/Y204) stimulation upon HEK293 cell treatment with EGF using the 2-plate assay protocol. All reagents were prepared according to each manufacturer's recommendations. Following cell treatment, cells were lysed with the corresponding kit's 1X Lysis Buffer supplemented with phosphatase inhibitors. Lysates were then tested on the same 384-well assay plate and according to the corresponding kit's standard protocol. The plate was read on an EnVision® (lamp excitation) following 4 hours of incubation. Data show that while the 2 TR-FRET assays exhibited comparable sensitivity (EC_{50} values), the THUNDER assay showed a higher S/B ratio.

THUNDER™ PHOSPHO-ERK1/2 (T202/Y204) ASSAY KIT



COMPANY B PHOSPHO-ERK1/2 (T202/Y204) ASSAY KIT

