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siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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TECHNICAL DATA SHEET

THUNDER™ Total CREB TR-FRET Cell Signaling Assay Kit

bioauxilium
BETTER TOOLS. REAL DISCOVERIES.

CATALOG NUMBERS

KIT-CREBT-100 (100 tests)
KIT-CREBT-500 (500 tests)
KIT-CREBT-2500 (2500 tests)
KIT-CREBT-5000 (5000 tests)
KIT-CREBT-10000 (10000 tests)

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

PRODUCT DESCRIPTION

This assay kit measures intracellular levels of **total CREB** 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 that recognize **total** (both phosphorylated and unphosphorylated) **total CREB**.

SPECIES REACTIVITY

Human, Mouse (Swiss-Prot Acc.: P16220; Entrez-Gene Id: 1385).

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

TR-FRET ASSAY PRINCIPLE

The **Total CREB** 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 **Total CREB** 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 **Total CREB** in the cell lysate. Data can be expressed as either the signal at 665 nm or the 665 nm/615 nm ratio.

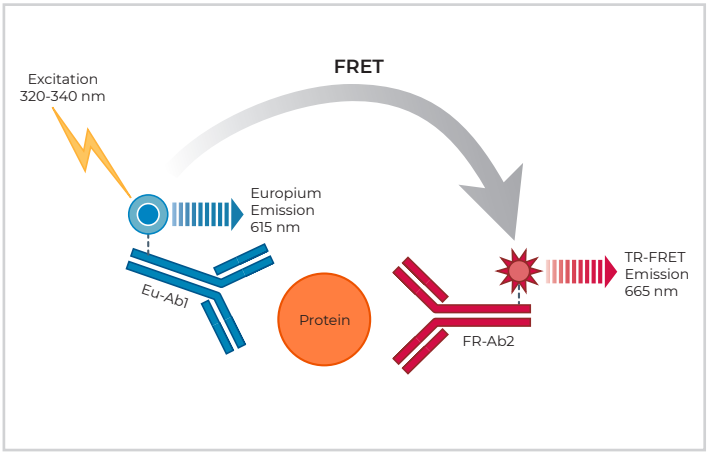


Figure 1 Schematic representation of the TR-FRET cell signaling assay principle.

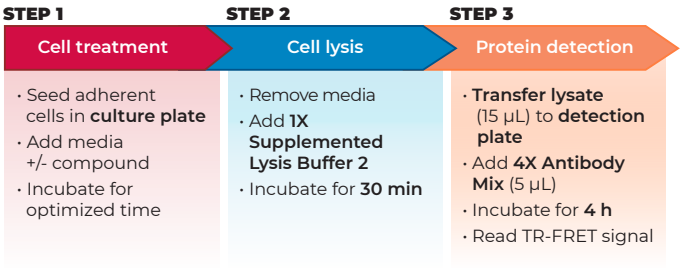


Figure 2 Assay workflow using the 2-plate (transfer) protocol.

KIT COMPONENTS

	100 points*	500 points*
Eu-labeled total-CREB antibody (Eu-Ab1)	5 µL	25 µL
Acceptor-labeled total-CREB antibody (FR-Ab2)	20 µL	100 µL
Lysis Buffer 2 (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

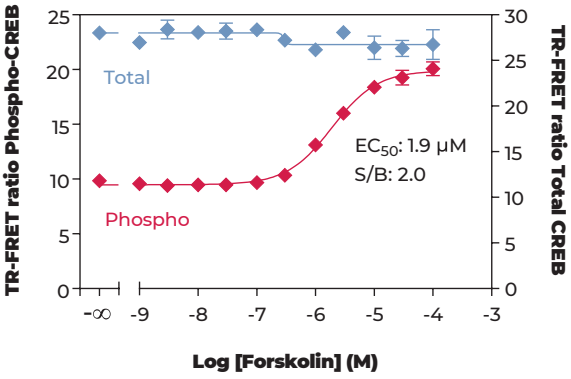
* The number of assay points is based on an assay volume of 20 µL in half-area 96-well or low-volume 384-well assay plates using the kit components at the recommended concentrations (refer to the User Manual).

VALIDATION DATA

This assay kit has been validated for the relative quantification of total CREB in A431, NIH3T3 and B lymphocyte cell lysates using the 2 plate assay protocol.

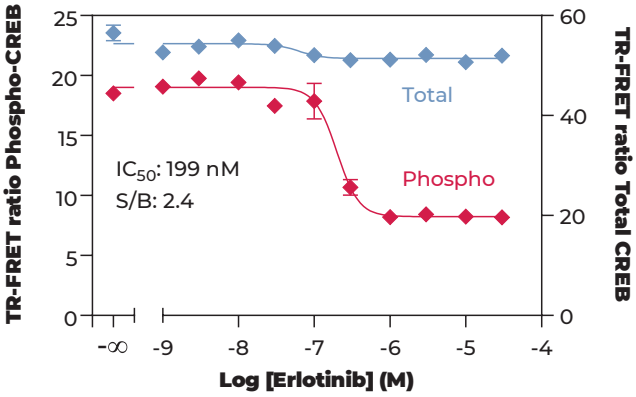
- Adherent cells were cultured overnight in a 96-well tissue culture plate before treatment, whereas B lymphocytes were centrifuged and resuspended at the desired density in RPMI without serum before treatment. Culture media used were: DMEM +10% FBS for A431, DMEM +10% CBS for NIH3T3, RPMI +15% FBS for B lymphocytes.
- For adherent cells, 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). For B lymphocytes, cells were lysed by adding 5X **Lysis buffer 2** (with phosphatase inhibitors), to a final concentration of 1X.
- 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 CREB.
- 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-CREB (S133) IN NIH3T3 CELLS



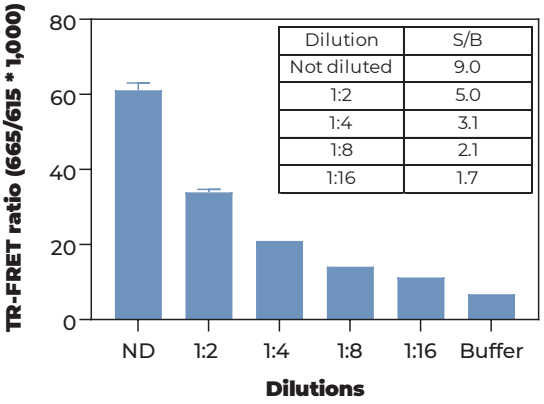
NIH3T3 cells (50,000 cells/well; in triplicate) were incubated with serial dilutions of Forskolin for 30 min at 37°C. Data show that treatment of NIH3T3 cells with Forskolin stimulates phosphorylation of CREB at S133 but does not affect the levels of total CREB.

INHIBITION OF PHOSPHO-CREB (S133) IN A431 CELLS



A431 cells (50,000 cells/well; in triplicate) were incubated with serial dilutions of the inhibitor Erlotinib for 30 min at 37°C. Cells were then stimulated with 1 nM of EGF for 10 min at 37°C. Data show that treatment of A431 cells with Erlotinib inhibits phosphorylation of CREB at S133 by EGF, but does not affect the levels of total CREB.

B LYMPHOCYTE CONTROL LYSATE TITRATION (QC TEST)



Dilution	S/B
Not diluted	9.0
1:2	5.0
1:4	3.1
1:8	2.1
1:16	1.7

Quality Control: the Total CREB assay kit is routinely tested against NECA-treated B lymphocyte lysates. B lymphocyte cells (80 millions in 8 mL of media) incubated in a T-flask with 300 nM of NECA (8 mL at 2X) for 30 min at RT. Following cell using 4 mL of 5X Lysis Buffer 2 (1X final), lysates were serially diluted with 1X Lysis Buffer 2 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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