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siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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TECHNICAL DATA SHEET

THUNDER™ Total BTK TR-FRET Cell Signaling Assay Kit

bioauxilium
BETTER TOOLS. REAL DISCOVERIES.

CATALOG NUMBERS

KIT-BTKT-100 (100 tests)
KIT-BTKT-500 (500 tests)
KIT-BTKT-2500 (2500 tests)
KIT-BTKT-5000 (5000 tests)
KIT-BTKT-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 BTK** 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) **BTK**.

SPECIES REACTIVITY

Human (Swiss-Prot Acc.: Q06187; Entrez-Gene Id: 695).

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

TR-FRET ASSAY PRINCIPLE

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

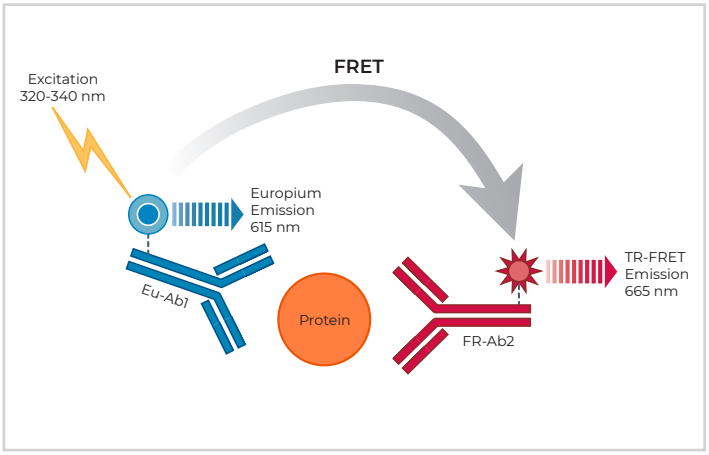


Figure 1 Schematic representation of the TR-FRET cell signaling assay principle.

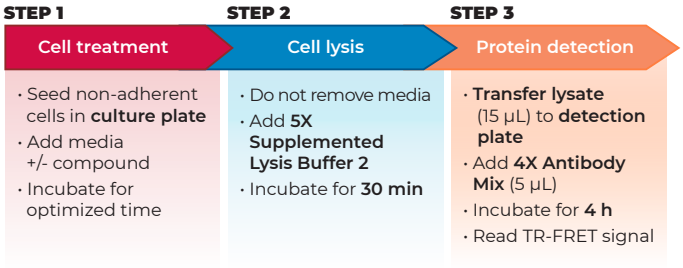


Figure 2 Assay workflow using the 2-plate (transfer) protocol.

KIT COMPONENTS

	100 points*	500 points*
Eu-labeled total-BTK antibody (Eu-Ab1)	5 µL	25 µL
Acceptor-labeled total-BTK 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

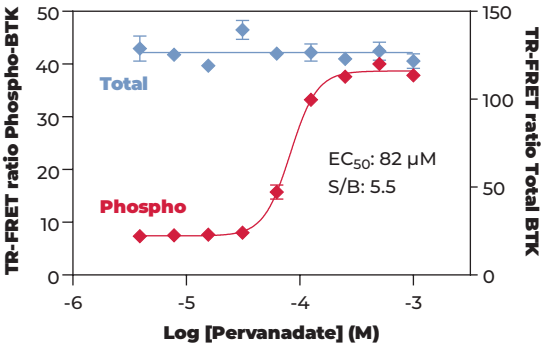
* The number of assay points is based on an assay volume of 20 µL in half-area 96-well or low-volume 384-well assay plates using the kit components at the recommended concentrations (refer to the User Manual).

VALIDATION DATA

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

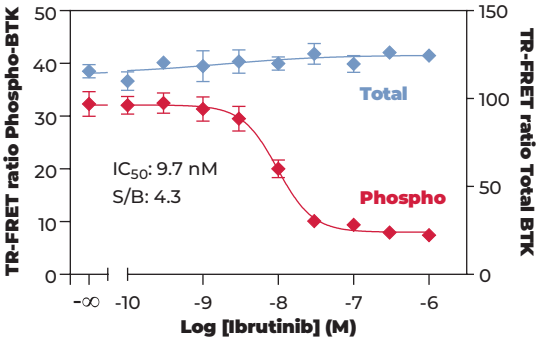
- Non-adherent cells were cultured in RPMI+10% FBS before being centrifuged and resuspended at the desired density in RPMI without serum.
- Following cell treatment, cells were lysed with the 5X **Lysis Buffer 2** (to a final concentration of 1X) supplemented with the 100X Phosphatase Inhibitor Cocktail diluted at 5X.
- 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 BTK.
- 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-BTK (Y223) IN RAJI CELLS



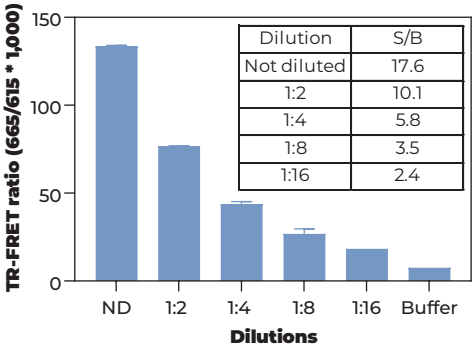
Raji cells (200,000 cells/well; in triplicate) were incubated with serial dilutions of Pervanadate for 30 min at 37°C. Data show that treatment of Raji cells with Pervanadate stimulates phosphorylation of BTK at Y223 but does not have a major effect on the levels of total BTK.

INHIBITION OF PHOSPHO-BTK (Y223) IN RAJI CELLS



Raji cells (200,000 cells/well; in triplicate) were incubated with serial dilutions of the inhibitor Ibrutinib for 60 min at 37°C. Cells were then stimulated with 150 µM Pervanadate for 30 min at 37°C. Data show that treatment of Raji cells with Ibrutinib inhibits phosphorylation of BTK at Y223 by Pervanadate, but does not have a major effect on the levels of total BTK.

RAJI CONTROL LYSATE TITRATION (QC TEST)



Dilution	S/B
Not diluted	17.6
1:2	10.1
1:4	5.8
1:8	3.5
1:16	2.4

Quality Control: the Total-BTK assay kit is routinely tested against Pervanadate treated Raji lysates. Raji cells were cultured, centrifuged, resuspended at 10 million cells/mL and stimulated with 200 µM of Pervanadate for 30 min at 37°C. Following cell lysis with 5X Lysis Buffer 2 (final concentration: 1X), 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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