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TECHNICAL DATA SHEET

THUNDER™ Total AKT pan TR-FRET Cell Signaling Assay Kit

bioauxilium
BETTER TOOLS. REAL DISCOVERIES.

CATALOG NUMBERS KIT-AKTT-100 (100 tests)
KIT-AKTT-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 **total AKT pan** 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) **AKT pan**.

SPECIES REACTIVITY

Human; Mouse (Swiss-Prot Acc. P31749, P31751, Q9Y243; Entrez Gene Id 207, 208 and 10000).

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

TR-FRET ASSAY PRINCIPLE

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

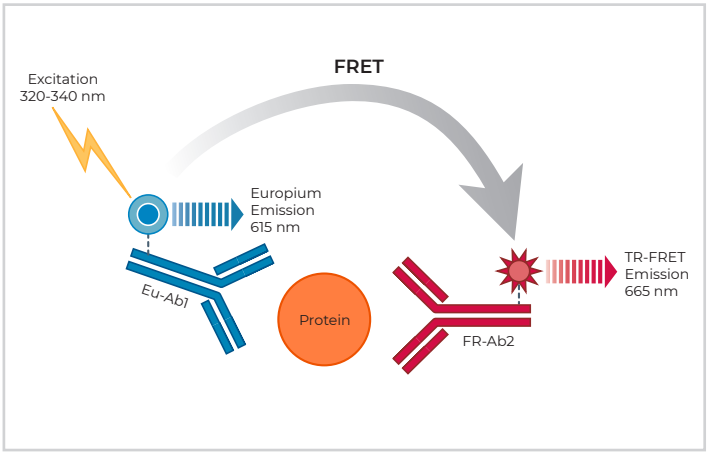


Figure 1 Schematic representation of the TR-FRET cell signaling assay principle.

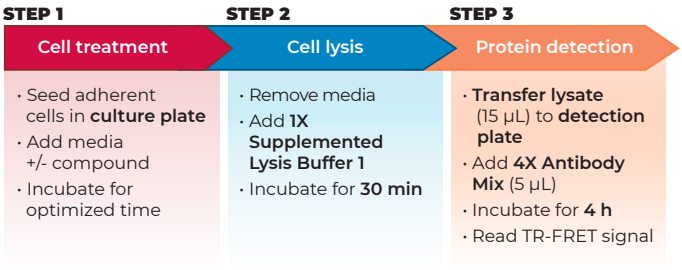


Figure 2 Assay workflow using the 2-plate (transfer) protocol.

KIT COMPONENTS

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

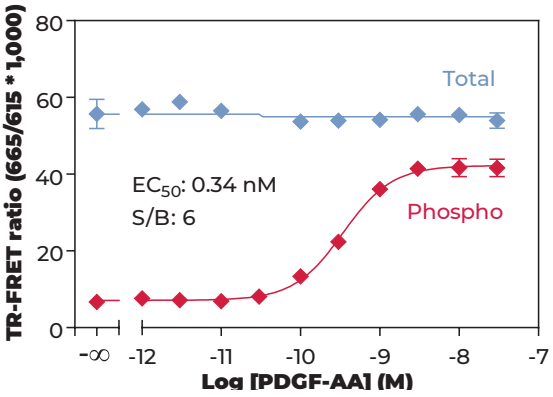
THIS PRODUCT IS NOT FOR RESALE OR DISTRIBUTION EXCEPT BY AUTHORIZED DISTRIBUTORS.

VALIDATION DATA

This assay kit has been validated for the relative quantification of total-AKT pan in NIH3T3 cell lysates using the 2-plate assay protocol.

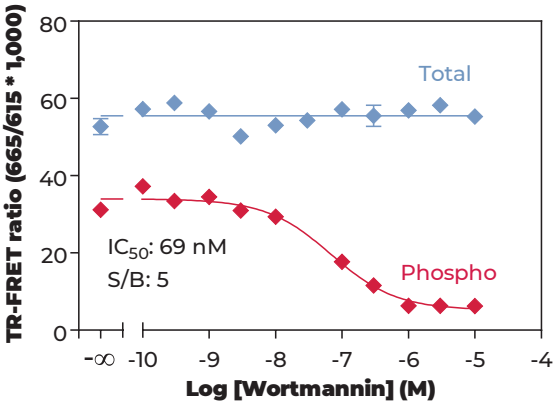
- Adherent cells were cultured 48 hours in a 96-well tissue culture plate, (DMEM + 10% CBS).
- Following cell treatment, the media was removed and cells were lysed with the 1X **Lysis Buffer 1** (50 µL) supplemented with the phosphatase inhibitors sodium fluoride (1 mM) and sodium orthovanadate (2 mM).
- 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-AKT pan.
- 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-AKT PAN (S473) IN NIH3T3 CELLS



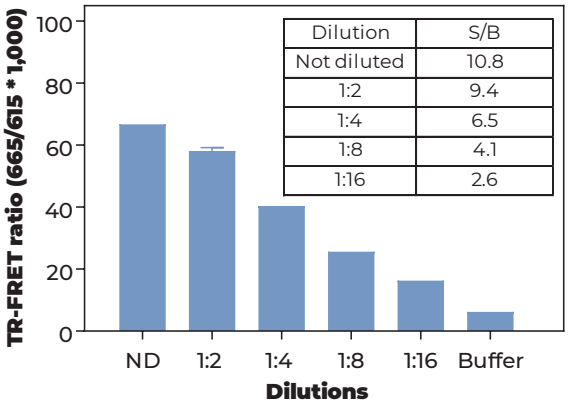
NIH3T3 cells (30,000 cells/well, in triplicate) were incubated with serial dilutions of PDGF-AA for 15 min at RT. Data show that treatment of NIH3T3 cells with PDGF-AA stimulates phosphorylation of AKT pan at S473 but does not affect the levels of total AKT pan.

INHIBITION OF PHOSPHO-AKT PAN (S473) IN NIH3T3 CELLS



NIH3T3 cells (30,000 cells/well, in triplicate) were incubated with serial dilutions of Wortmannin for 30 min at RT. Cells were then stimulated with 1 nM PDGF-AA for 15 minutes at RT. Data show that treatment of NIH3T3 cells with Wortmannin inhibits phosphorylation of AKT pan at S473 by PDGF-AA but does not affect the levels of total AKT pan.

NIH3T3 CONTROL LYSATE TITRATION (QC TEST)



Dilution	S/B
Not diluted	10.8
1:2	9.4
1:4	6.5
1:8	4.1
1:16	2.6

Quality Control: the Total AKT pan assay kit is routinely tested against PDGF-AA treated NIH3T3 lysates. NIH3T3 cells were cultured in a T175 flask to 95% confluence and stimulated with 3 nM of PDGF-AA for 15 min at RT. Following cell lysis using 4 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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