TECHNICAL DATA SHEET

THUNDER™ Total VAV1
TR-FRET Cell Signaling Assay Kit

bioauxilium
BETTER TOOLS. REAL DISCOVERIES.

CATALOG NUMBERS

KIT-VAV1-100 (100 tests)
KIT-VAV1-500 (500 tests)
KIT-VAV1-2500 (2500 tests)
KIT-VAV1-5000 (5000 tests)
KIT-VAV1-10000 (10000 tests)

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

Visit Product Page

PRODUCT DESCRIPTION

This assay kit measures intracellular levels of **total VAV1** 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 VAV1**.

SPECIES REACTIVITY

Human (Swiss-Prot Acc.: P15498; Entrez-Gene Id: 7409).

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

TR-FRET ASSAY PRINCIPLE

The **Total VAV1** 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 VAV1** 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 VAV1** in the cell lysate. Data can be expressed as either the signal at 665 nm or the 665 nm/615 nm ratio.

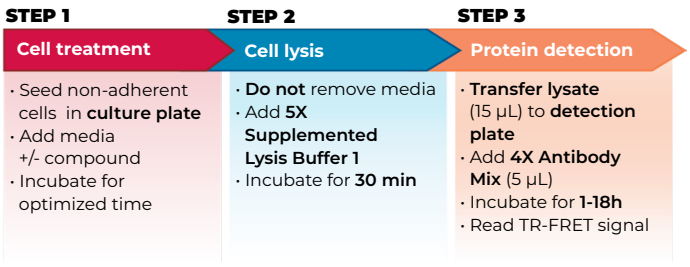


Figure 2 Assay workflow using the 2-plate (transfer) protocol.

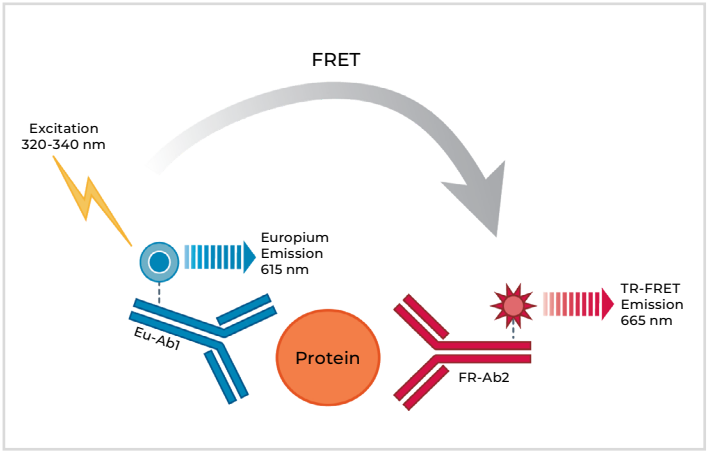


Figure 1 Schematic representation of the TR-FRET cell signaling assay principle.

KIT COMPONENTS

	100 points*	500 points*
Eu-labeled total VAV1 antibody (Eu-Ab1)	5 µL	25 µL
Acceptor-labeled total VAV1 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
Phosphatase Inhibitor Cocktail (100X)	50 µL	250 µL

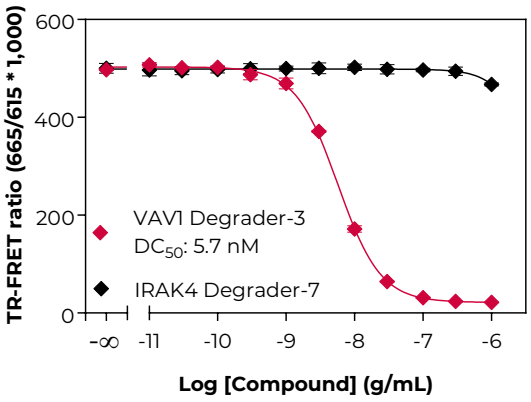
* The number of assay points is based on an assay volume of 20 µL in half-area 96-well or low-volume 384-well assay plates using the kit components at the recommended concentrations (refer to the User Manual).

VALIDATION DATA

This assay kit has been validated for the relative quantification of total VAV1 in Jurkat cell lysates using the 2-plate assay protocol.

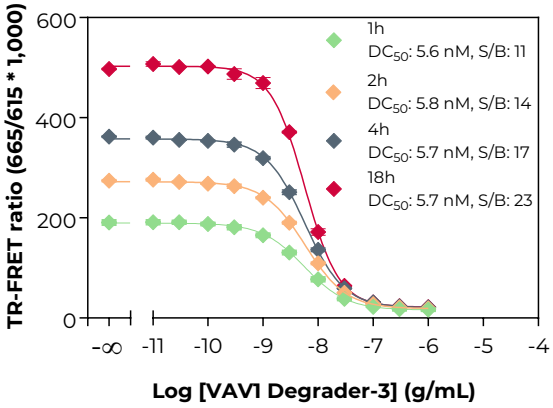
- Non-adherent cells were cultured in RPMI+10% FBS before being centrifuged and resuspended at the desired density in RPMI without serum.
- Following cell treatment, cells were lysed with the 5X **Lysis Buffer 1** (to a final concentration of 1X).
- Following a **30-min** incubation at room temperature (RT) on an orbital shaker (400 rpm), lysates (15 µL) were 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 VAV1.
- The plate was incubated at RT for **18 hours** (unless otherwise indicated) and the TR-FRET signal was recorded at 665 and 620 nm (PHERAstar® *FSX*; laser excitation).

DEGRADATION OF VAV1 IN JURKAT CELLS



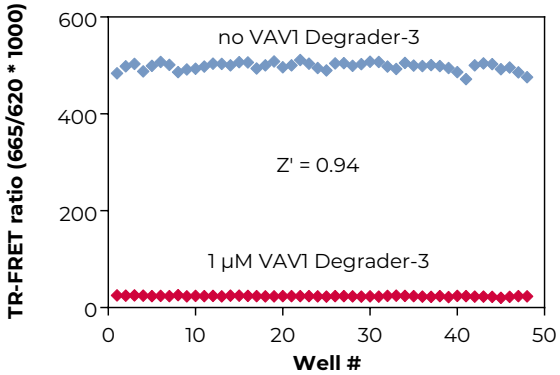
Jurkat cells (200,000 cells/well; in triplicate) were incubated with serial dilutions of the molecular glues VAV1 Degrader-3 and IRAK4 Degrader-7 (as a negative control), for 24 hours at 37°C. Data show that treatment of Jurkat cells with VAV1 Degrader-3 degrades total VAV1, whereas VAV1 levels were not modulated by IRAK4 Degrader-7.

DEGRADATION OF VAV1 (TIME COURSE)



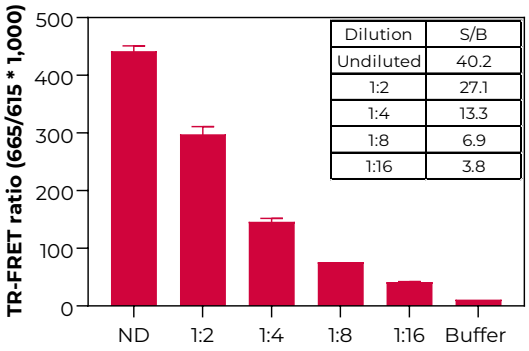
Jurkat cells (200,000 cells/well; in triplicate) were incubated with serial dilutions of the molecular glue VAV1 Degrader-3 for 24 hours at 37°C. Data show that treatment of Jurkat cells with VAV1 Degrader-3 degrades total VAV1 and that robust S/B ratio and pharmacology can be obtained after 1h of incubation.

Z'-FACTOR DETERMINATION



Jurkat cells (200,000 cells/well; in triplicate) were incubated with or without 1 µM of VAV1 Degrader-3 for 24 hours at 37°C. The Z' factor value was determined after an 18-hour incubation period, using a total of 48 wells for each treatment group. The Z'-factor value of 0.94 indicates that the assay is robust and suitable for HTS.

JURKAT CONTROL LYSATE TITRATION (QC TEST)



Dilution	S/B
Undiluted	40.2
1:2	27.1
1:4	13.3
1:8	6.9
1:16	3.8

Quality Control: the total VAV1 assay kit is routinely tested against Jurkat lysates. Jurkat cells were cultured in a T175 flask, centrifuged and resuspended at 7.5 million cells/mL. Following cell lysis using 5X Lysis Buffer 1 (final concentration: 1X), 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.