TECHNICAL DATA SHEET

THUNDER™ Total GAPDH TR-FRET Cell Signaling Assay Kit

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CATALOG NUMBERS KIT-GAPDHT-100 (100 tests)
KIT-GAPDHT-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 GAPDH** 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) **GAPDH**.

SPECIES REACTIVITY

Human, Mouse (Swiss-Prot Acc.: P04406; Entrez-Gene Id: 2597).

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

TR-FRET ASSAY PRINCIPLE

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

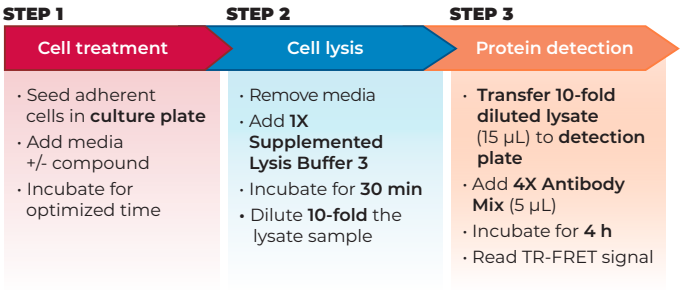


Figure 2 Assay workflow using the 2-plate (transfer) protocol.

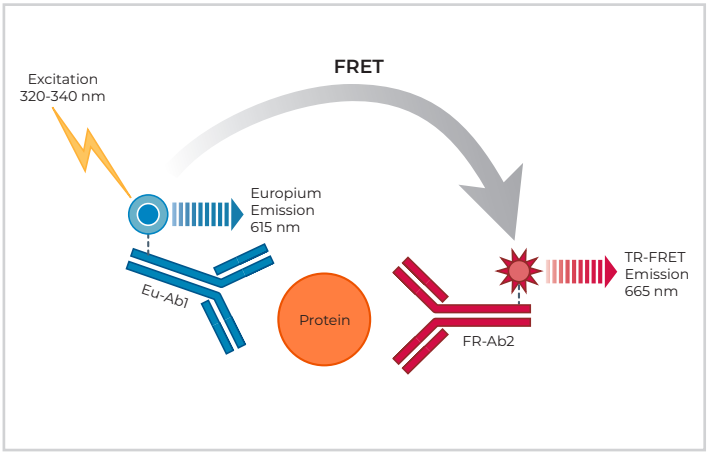


Figure 1 Schematic representation of the TR-FRET cell signaling assay principle.

KIT COMPONENTS

	100 points*	500 points*
Eu-labeled total-GAPDH antibody (Eu-Ab1)	5 µL	25 µL
Acceptor-labeled total-GAPDH antibody (FR-Ab2)	20 µL	100 µL
Lysis Buffer 3 (5X)	1 mL	5 mL
Detection Buffer (10X)	50 µL	250 µL
Positive control cell lysate	100 µL	500 µL

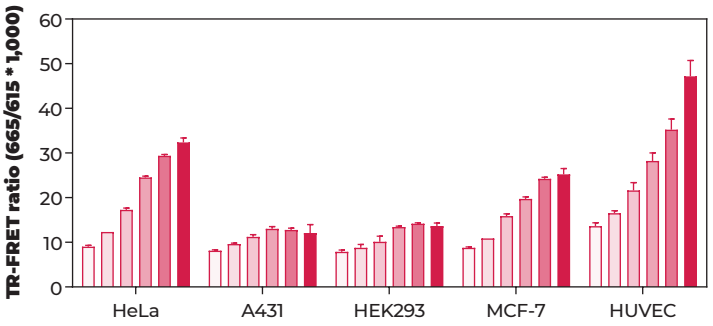
* The number of assay points is based on an assay volume of 20 µL in half-area 96-well or low-volume 384-well assay plates using the kit components at the recommended concentrations (refer to the User Manual).

VALIDATION DATA

This high-sensitivity assay kit has been validated for the relative quantification of total GAPDH in several cell lines lysates using the 2 plate assay protocol.

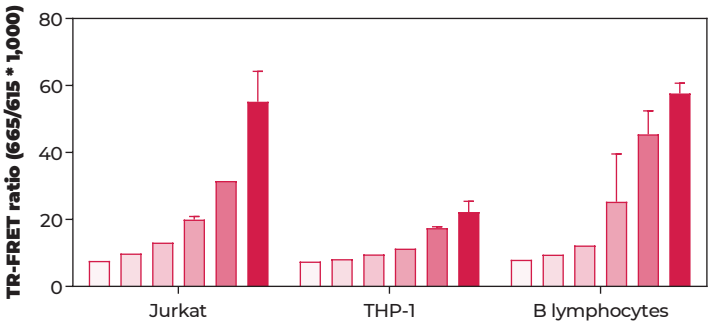
- Adherent cells were cultured overnight in a 96-well tissue culture plate. Suspension cells were centrifuged and resuspended at the desired density.
- Cells were lysed in 1X **Lysis Buffer 3** supplemented with the phosphatase inhibitors sodium fluoride (1 mM) and sodium orthovanadate (2 mM).
- Following a **30-min** incubation at room temperature (RT) on an orbital shaker (400 rpm), **lysates were diluted 10-fold in 1X Lysis Buffer 3.**
- **Diluted 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-GAPDH.
- The plate was incubated at RT for **4 hours** and the TR-FRET signal was recorded at 665 and 615 nm (EnVision®; lamp excitation).

ADHERENT CELLS



Note that similar data can be obtained with Lysis Buffer 4.

SUSPENSION CELLS



Note that lysates from different cells must be tested neat and diluted in 1X supplemented Lysis Buffer to ensure that samples are within the assay linear range.

DETECTION OF GAPDH BY WESTERN BLOT

Western blot shows lysates of:
1. MCF7;
2. HeLa;
3. Jurkat;
4. HEK293;
5. Recombinant GAPDH (100 ng);
6. Negative control: GLP-1(7-36) amide (100 ng).

A specific band was detected for GAPDH at approximately 37 kDa.

FOR MORE INFORMATION ON DEVELOPING AND OPTIMIZING TR-FRET CELL SIGNALING ASSAYS, CONSULT THE USER MANUAL.

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