TECHNICAL DATA SHEET

THUNDER™ Total Rb
TR-FRET Cell Signaling Assay Kit

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CATALOG NUMBERS

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

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

Visit Product Page

PRODUCT DESCRIPTION

This High Performance assay kit measures intracellular levels of **total Rb** 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) **Rb**.

SPECIES REACTIVITY

Human (Swiss-Prot Acc.: P06400; Entrez-Gene Id: 5925).

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

TR-FRET ASSAY PRINCIPLE

The **total Rb** 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 3 provided in the kit. Then **total Rb** 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 Rb** in the cell lysate. Data can be expressed as either the signal at 665 nm or the 665 nm/615 nm ratio.

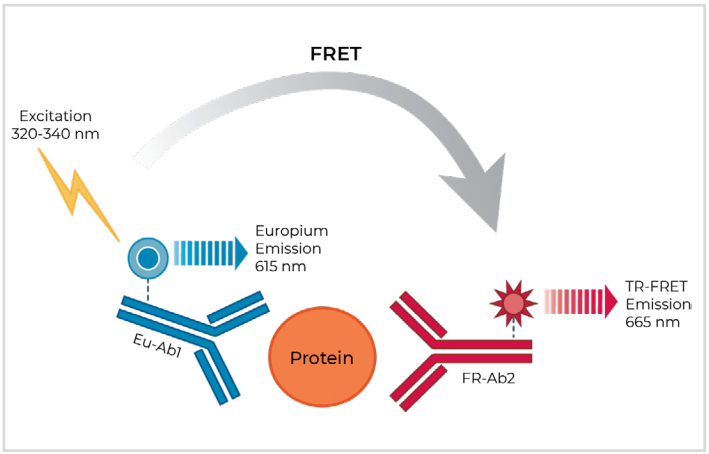


Figure 1 Schematic representation of the TR-FRET cell signaling assay principle.

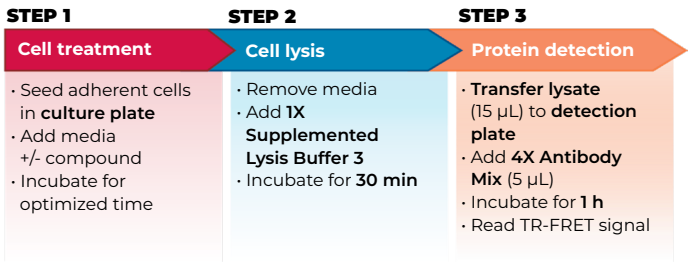


Figure 2 Assay workflow using the 2-plate (transfer) protocol.

KIT COMPONENTS

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

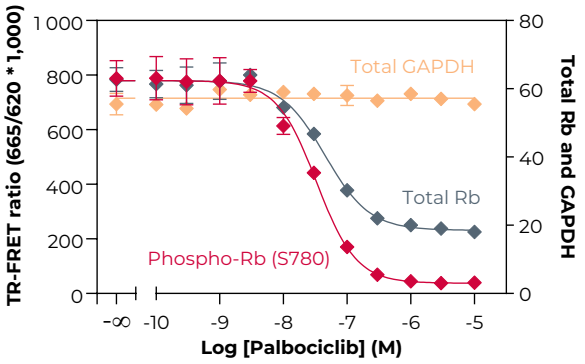
* The number of assay points is based on an assay volume of 20 µL in half-area 96-well or low-volume 384-well assay plates using the kit components at the recommended concentrations (refer to the User Manual).

VALIDATION DATA

This assay kit has been validated for the relative quantification of total Rb in HT-29 cell lysates using the 2-plate assay protocol.

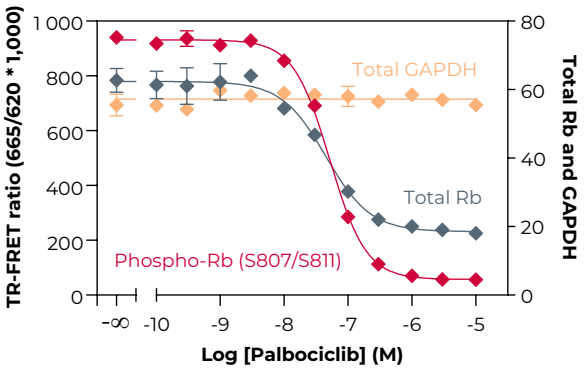
- Adherent cells were plated at 40K cells/well and incubated for 7 hours in a 96-well tissue culture plate (McCoy + 10% FBS), followed by a 24 hours starvation (McCoy without serum).
- Following cell treatment, the media was removed and cells were lysed with the 1X **Lysis Buffer 3** (50 µL) supplemented with the 100X Phosphatase Inhibitor Cocktail diluted at 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 Rb.
- The plate was incubated at RT for **1 hour** and the TR-FRET signal was recorded at 665 and 620 nm (PHERASTAR® FSX; laser excitation).

INHIBITON OF PHOSPHO-RB (S780) IN HT-29 CELLS



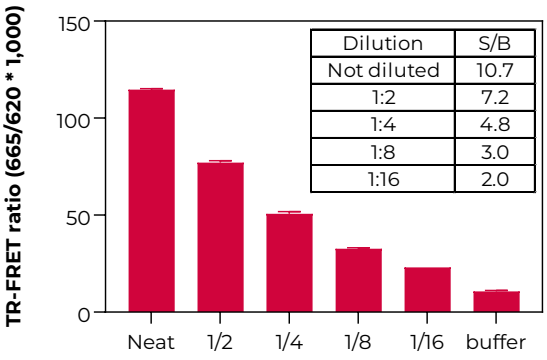
HT-29 cells (40,000 cells/well; in triplicate) were starved for 24 hours and then incubated with serial dilutions of Palbociclib for 20 hours at 37°C, 5% CO_2. Data show that treatment of HT-29 cells with palbociclib inhibits both total Rb and phospho-Rb (S780). However, levels of housekeeping total GAPDH (measured as a control) remain stable.

INHIBITON OF PHOSPHO-RB (S807/S811) IN HT-29 CELLS



HT-29 cells (40,000 cells/well; in triplicate) were starved for 24 hours and then incubated with serial dilutions of Palbociclib for 20 hours at 37°C, 5% CO_2. Data show that treatment of HT-29 cells with palbociclib inhibits both total Rb and phospho-Rb (S807/S811). However, levels of housekeeping total GAPDH (measured as a control) remain stable.

HT-29 CONTROL LYSATE TITRATION (QC TEST)



Dilution	S/B
Not diluted	10.7
1:2	7.2
1:4	4.8
1:8	3.0
1:16	2.0

Quality Control: the total Rb assay kit is routinely tested against HT-29 lysates. HT-29 cells are cultured in a T175 flask to 90% confluence. Following cell lysis using 1X Lysis Buffer 3, lysates were serially diluted with 1X Lysis Buffer 3 and tested in triplicate. Data show a linear relationship between lysate dilutions and TRFRET ratio values.

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