

TECHNICAL DATA SHEET



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THUNDER™ Phospho-Rb (S780) + Total Rb TR-FRET Cell Signaling Assay Kit

CATALOG NUMBERS KIT-RBS780PT-500
400 points for phospho-Rb
and 100 points for total Rb

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 **Phospho-Rb (S780) and 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 antibody pairs, one that recognizes **Rb** phosphorylated at **Ser780**, and another that recognizes 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 **Phospho-Rb (S780) + 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 provided in the kit. Then **Phospho-Rb (S780) + 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 matched labeled antibodies to distinct epitopes on the target protein (either **phospho-Rb** or **total Rb**) 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 **Phospho-Rb (S780)** and **Total Rb** in the cell lysate. Data can be expressed as either the signal at 665 nm or the 665 nm/615 nm ratio.

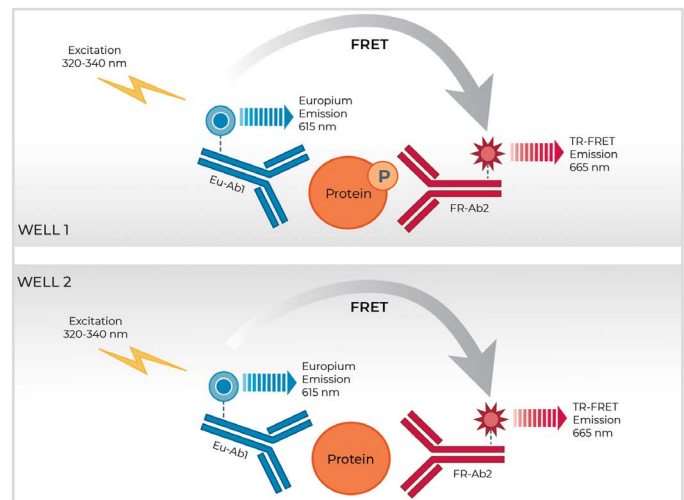


Figure 1 Schematic representation of the TR-FRET cell signaling assay principle.

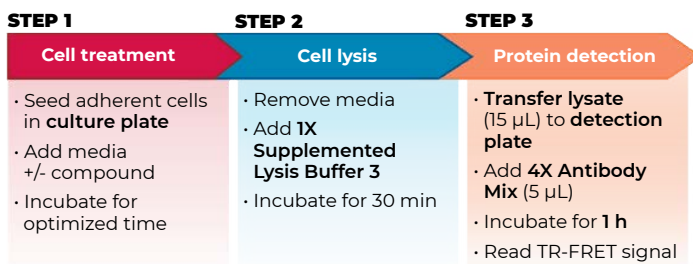


Figure 2 Assay workflow using the 2-plate (transfer) protocol.

KIT COMPONENTS

	500 points*
Eu-labeled phospho-Rb (S780) antibody (Eu-Ab1)	20 µL
Acceptor-labeled phospho-Rb (S780) antibody (FR-Ab2)	80 µL
Eu-labeled total-Rb antibody (Eu-Ab3)	5 µL
Acceptor-labeled total-Rb antibody (FR-Ab4)	20 µL
Lysis Buffer 3 (5X)	5 mL
Detection Buffer (10X)	250 µL
Positive control cell lysate	200 µL
Phosphatase Inhibitor Cocktail (100X)	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).

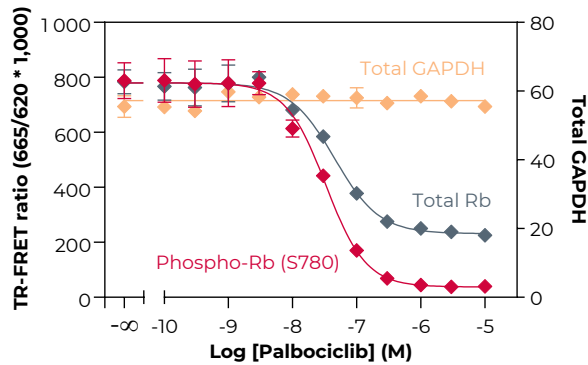
THIS PRODUCT IS NOT FOR RESALE OR DISTRIBUTION EXCEPT BY AUTHORIZED DISTRIBUTORS.

VALIDATION DATA

This assay kit has been validated for the relative quantification of phospho-Rb (S780) and total Rb in HT-29 cell lysates using the 2-plate assay protocol.

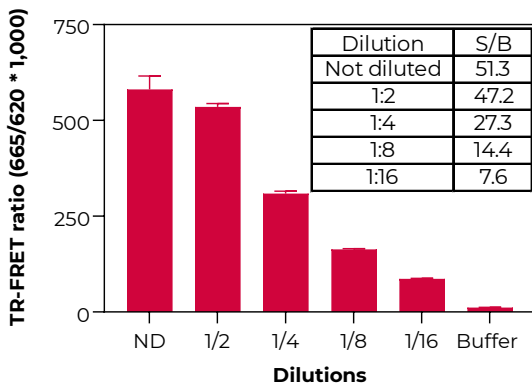
- Adherent cells were cultured overnight in a 96-well tissue culture plate (McCoy's 5A + 10% FBS).
- Following cell treatment, the media was removed and cells were lysed with the 1X Lysis Buffer 3 (50 μ L) supplemented with the 100X Phosphatase Inhibitor Cocktail diluted at 1X.
- Following a **30-min** incubation at room temperature (RT) on an orbital shaker (400 rpm), lysates (15 μ L) were then transferred to a 384-well assay plate followed by addition of the labeled antibodies Eu-Ab1 and FR-Ab2 (5 μ L) for detection of phospho-Rb (S780) or Eu-Ab3 and FR-Ab4 (5 μ L) for detection of total Rb.
- The plate was incubated at RT for **1 hour** and the TR-FRET signal was recorded at 665 and 615 nm (PHERAstar® FSX; laser excitation).

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

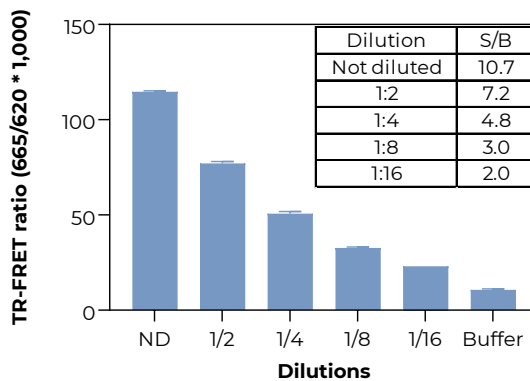


HT-29 cells (40,000 cells/well; in triplicate) were starved for 24 hours and then incubated with serial dilutions of Palbociclib for 20 hours at 37°C, 5% CO₂. Data show that treatment of HT-29 cells with palbociclib inhibits both total Rb and phospho-Rb (S780). However, levels of house-keeping total GAPDH (measured as a control) remain stable.

HT-29 CONTROL LYSATE TITRATION (QC TEST) PHOSPHO-RB (S780)



HT-29 CONTROL LYSATE TITRATION (QC TEST) TOTAL RB



Quality Control: the Phospho-Rb (S780) and Total Rb assay kit is routinely tested against HT-29 lysates. HT-29 cells were cultured in a T175 flask to 90% confluence. Following cell lysis using 5 mL of 1X Lysis Buffer 3 supplemented with Phosphatase Inhibitor Cocktail, lysates were serially diluted with 1X Lysis Buffer 3 and tested in triplicate. Data show a linear relationship between lysate dilutions and TR-FRET ratio values.

