

TECHNICAL DATA SHEET

THUNDER™ Total CDK6 TR-FRET Cell Signaling Assay Kit



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BETTER TOOLS. REAL DISCOVERIES.

CATALOG NUMBERS KIT-CDK6-100 (100 tests)
KIT-CDK6-500 (500 tests)
KIT-CDK6-2500 (2500 tests)
KIT-CDK6-5000 (5000 tests)
KIT-CDK6-10000 (10000 tests)

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

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PRODUCT DESCRIPTION

This assay kit measures intracellular levels of **total CDK6** 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 CDK6**.

SPECIES REACTIVITY

Human
Swiss-Prot Acc.:Q00534
Entrez-Gene Id: 1021
Other species should be tested on a case-by-case basis.

TR-FRET ASSAY PRINCIPLE

The **Total CDK6** 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 CDK6** 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 CDK6** in the cell lysate. Data can be expressed as either the signal at 665 nm or the 665 nm/615 nm ratio.

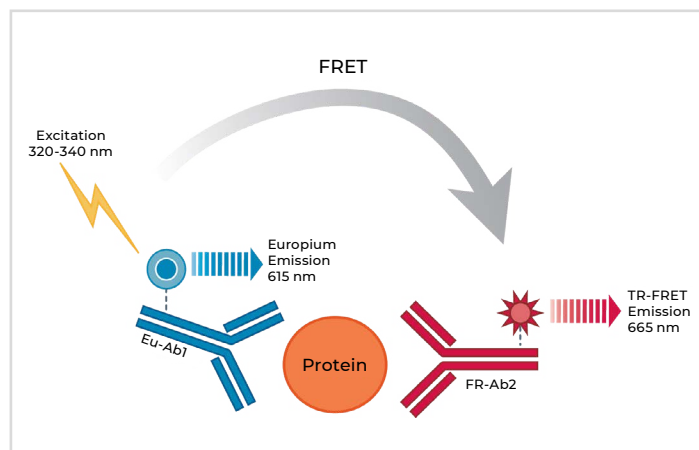


Figure 1 Schematic representation of the TR-FRET cell signaling assay principle.

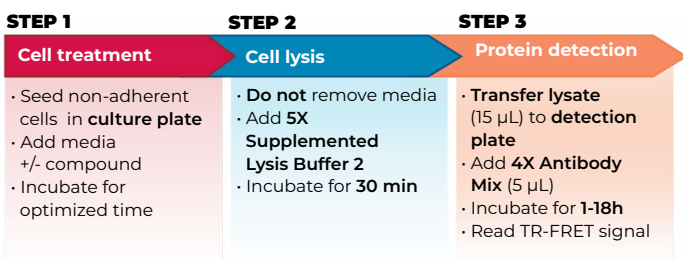


Figure 2 Assay workflow using the 2-plate (transfer) protocol.

KIT COMPONENTS

	100 points*	500 points*
Eu-labeled total CDK6 antibody (Eu-Ab1)	5 µL	25 µL
Acceptor-labeled total CDK6 antibody (FR-Ab2)	20 µL	100 µL
Lysis Buffer 2 (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

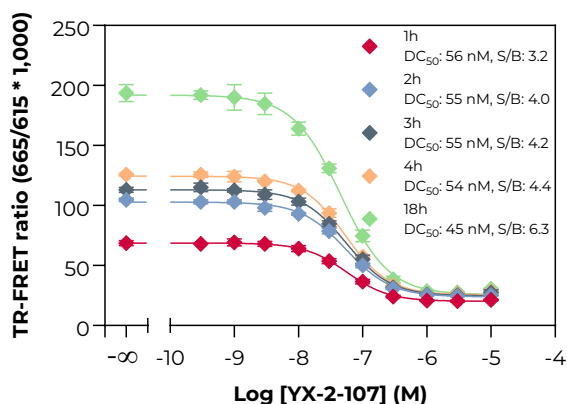
* The number of assay points is based on an assay volume of 20 µL in half-area 96-well or low-volume 384-well assay plates using the kit components at the recommended concentrations (refer to the User Manual).

VALIDATION DATA

This assay kit has been validated for the relative quantification of total CDK6 in Jurkat cell lysates using the 2-plate assay protocol.

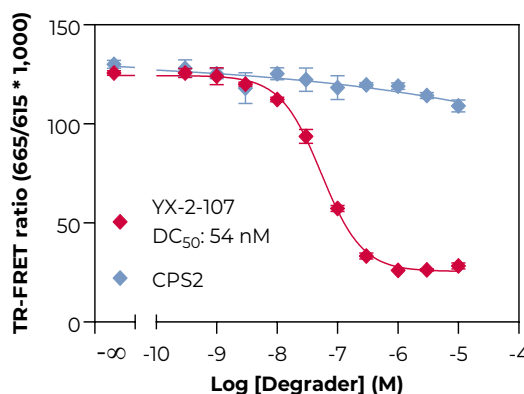
- Non-adherent cells were cultured in RPMI+10% FBS before being centrifuged and resuspended at the desired density in RPMI without serum.
- Following cell treatment, cells were lysed with the 5X **Lysis Buffer 2** (to a final concentration of 1X).
- Following a **30-min** incubation at room temperature (RT) on an orbital shaker (400 rpm), lysates (15 μ L) were 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 total CDK6.
- The plate was incubated at RT for **4 hours** (unless otherwise indicated) and the TR-FRET signal was recorded at 665 and 620 nm (PHERAstar® FSX; laser excitation).

DEGRADATION OF CDK6 IN JURKAT CELLS



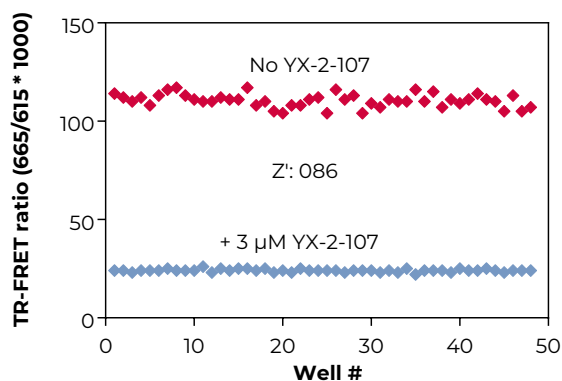
Jurkat cells (25,000 cells/well; in triplicate) were incubated with serial dilutions of the PROTAC degrader YX-2-107, for 4 hours at 37°C. Data show that treatment of Jurkat cells with YX-2-107 degrades total CDK6 and that robust S/B ratio and pharmacology can be obtained after 1h of incubation.

EFFECT OF TWO PROTAC DEGRADERS ON CDK6



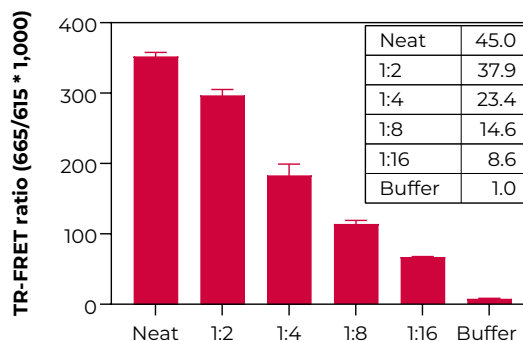
Jurkat cells (25,000 cells/well; in triplicate) were incubated with serial dilutions of the PROTAC degrader YX-2-107 and CPS2 (a selective CDK2 degrader) for 4 hours at 37°C. Data obtained after 4 hours of incubation showed that treatment with YX-2-107 induced degradation of total CDK6 in Jurkat cells, whereas CDK6 levels were not affected by CPS2 treatment.

Z'-FACTOR DETERMINATION



Jurkat cells (25,000 cells/well; in triplicate) were incubated without or with 3 μ M of PROTAC degrader YX-2-107 for 4 hours at 37°C. The Z' factor value was determined after a 4h-incubation time, using a total of 48 wells for each treatment group. The Z' factor value of 0.86 indicates that the assay is robust and suitable for HTS.

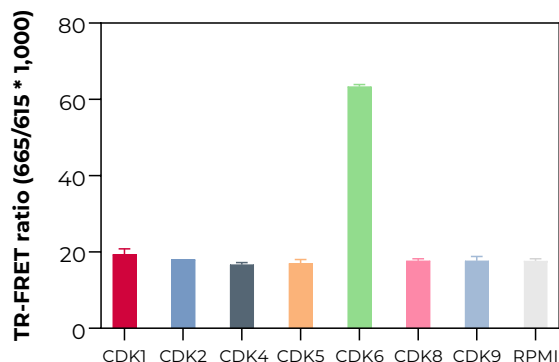
JURKAT CONTROL LYSATE TITRATION (QC TEST)



Quality Control: the total CDK6 assay kit is routinely tested against Jurkat lysates. Jurkat cells were cultured in a T175 flask, centrifuged and resuspended at 5 million cells/mL. Following cell lysis using 5X Lysis Buffer 2 (final concentration: 1X), lysates were serially diluted with 1X Lysis Buffer 2 and tested in triplicate. Data show a linear relationship between lysate dilutions and TR-FRET ratio values.

VALIDATION DATA (CONTINUED)

ASSAY SPECIFICITY



Specificity of the assay was evaluated using recombinant CDK1, CDK2, CDK4, CDK5, CDK6, CDK8, and CDK9 proteins, each at a concentration of 300 ng/mL, demonstrating specific detection of CDK6 with no detectable cross-reactivity toward the other CDKs tested. TR-FRET signal was recorded after 4 hours of incubation.

