CyQUANT™ Direct Red Cell Proliferation Assay Kit

Catalog Numbers C35013 and C35013C

Pub. No. MAN0018644 Rev. B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The CyQUANT™ Direct Red Cell Proliferation Assay Kit is used to perform fluorescence-based proliferation and cytotoxicity assays using microplate readers. The kit is designed for use with multi-well plates (96-, 384-, or 1,536-well plate formats), making it ideal for high-throughput screening applications. The assay is performed with mammalian cells, either adherent or in suspension, and has a linear detection range for most cell types. The assay can also be made quantitative by creating a standard curve of known cell numbers.

Cell growth, viability (Figure 1), or compound toxicity (Figure 2) is measured using a simple, no wash, add-mix-read assay which can be completed in one hour. The excitation and emission wavelengths (Figure 3) of the red dye make it ideal for multiplexing with green fluorescent dyes or proteins, and the fluorescence signal is stable for several hours (Figure 4). For more information on the assay, see “About the CyQUANT™ Direct Red Cell Proliferation Assay” on page 2.

Contents and storage

Item	C35013 (10 x 96-well plates)	C35013C (100 x 96-well plates)	Storage[1]
CyQUANT™ Direct Red nucleic acid stain (Component A)	0.5 mL in DMSO	5 mL in DMSO	Store at 2°C to 25°C, or ≤–20°C for long term Desiccate Protect from light
CyQUANT™ Direct Red background suppressor (Component B)	2.5 mL in ultrapure water	25 mL in ultrapure water	
Peak fluorescence excitation/emission maxima 622 nm/645 nm when bound to nucleic acid.			

[1] When stored as directed, the kit is stable for 6 months from the date of receipt.

Before you begin

Prepare cells for viability/proliferation assay

1. Plate cells into the wells of a microtiter plate.
2. Grow the cells for the required amount of time for your experiment, or perform the assay immediately.

Prepare cells for cytotoxicity assay

1. Plate cells into the wells of a microtiter plate.
2. Add the test compound(s).
3. Incubate cells with the test compound(s).

Prepare 2X Detection Reagent

1. Prepare 12 mL of 2X Detection Reagent by adding the following components in a 15-mL tube.

Component	Volume
Cell culture media (with or without serum)	11.7 mL
CyQUANT™ Direct Red nucleic acid stain	48 µL
CyQUANT™ Direct Red background suppressor	240 µL

12 mL of 2X Detection Reagent is sufficient for filling one microtiter plate with 100 µL/well in a 96-well plate, 25 µL/well in a 384-well plate, or 5 µL/well in a 1,536-well plate.

2. Mix the 2X Detection Reagent well and keep at room temperature until ready for use.

Perform CyQUANT™ Direct Red Cell Proliferation Assay

1. Add an equal volume of 2X Detection Reagent to cells in culture.
2. Incubate cells with the Detection Reagent for 60 minutes at 37°C.
3. Read fluorescence of samples using filter sets or appropriate wavelengths (i.e., 622/645 nm).

   Note: A bottom-read microplate reader is required to detect the fluorescence signal in the presence of the background suppression reagent. Use caution when moving microtiter plates to allow suspension cells to remain settled or in contact with the bottom of the plate.

(*Optional*) Construct a standard curve

Create a reference standard curve if the goal is to determine the percentage of live cells. The cell number dilution allows for the conversion of the sample fluorescence values into cell numbers.

1. Generate a dilution series with the same cell type used in the experiment in the wells of a microtiter plate. Use cell culture media to create dilutions corresponding to cell numbers ranging from 50 to 50,000 cells in 25 µL (for a 384-well plate), or 100 µL (for a 96-well plate) volumes. Include a sample with no cells as a negative control.
2. Proceed with detection by following the experimental protocol as outlined above.

About the CyQUANT™ Direct Red Cell Proliferation Assay

The CyQUANT™ Direct Red Cell Proliferation assay consists of a cell-permeant DNA-binding red fluorescent probe and a cell-impermeant background suppressor that are mixed and added to cells in a fluorescence microplate. The cell-permeant DNA-binding stain is a live cell permeable reagent that enters all cells, but concentrates mainly in the nucleus. The cell-impermeant background suppressor is impermeable in live cells, and only enters dead cells or cells with compromised cell membranes. Upon entering dead and dying cells the background suppressor suppresses the signal from the fluorescent probe, thus eliminating the need to perform wash steps. The end result is that fluorescence from healthy cells is detected by microplate reader (see Figure 3 for fluorescence spectra). Maximal fluorescence intensity of the readout is obtained within 60 minutes after reagent addition and the signal is stable for several hours (Figure 4), affording work-flow convenience.

The combination of these two components results in an assay based on both DNA content and membrane integrity. Because cells are not lysed or permeabilized for the assay, and because staining does not require active metabolism, the readout provides a direct measure of cell proliferation and viability. The CyQUANT™ Direct Red Cell Proliferation Assay can therefore be used to assess cell growth, cell viability, or compound toxicity in a range of applications, from high-throughput screening to bioproduction.

Since the amount of cellular DNA is highly regulated, detection methods based on determining the amount of DNA are highly accurate. Also, because fluorescence intensity is linearly dependent on cell number, the assay can be made quantitative by using a standard curve of known cell numbers.

The results of the assay are highly linear as demonstrated by plotting a cellular dilution series against fluorescence intensity for both CyQUANT™ Direct Red and CyQUANT™ Direct assays (Figure 1).

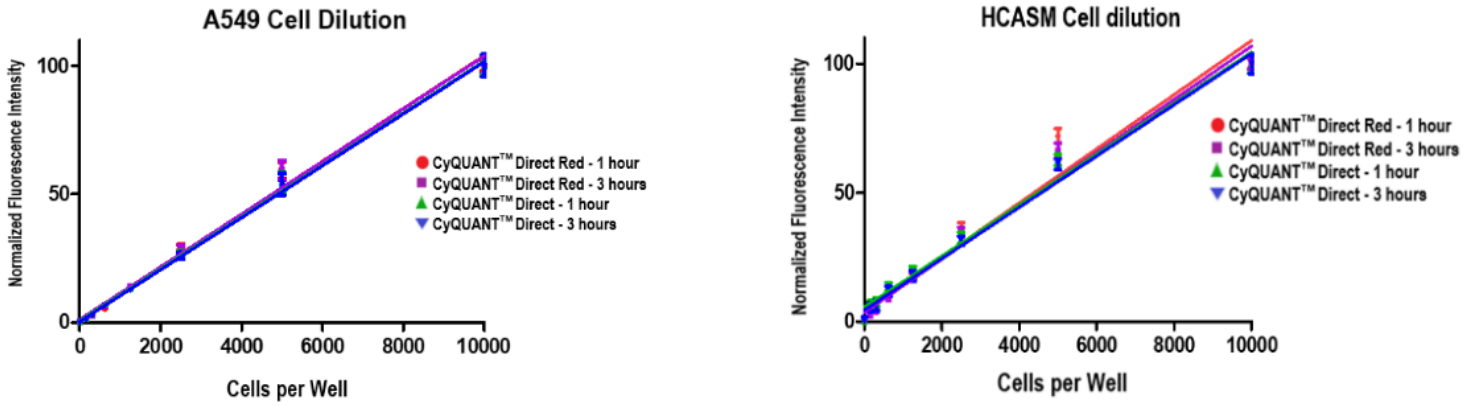

Figure 1 Consistent results using CyQUANT™ Direct Red and CyQUANT™ Direct Cell Proliferation Assays

A day before the proliferation assay, a serial dilution of A549 of HCASM (human coronary artery smooth muscle) cells was added into a 96-well plate. After an overnight incubation, CyQUANT™ Direct Red or CyQUANT™ Direct was added to the wells following the corresponding protocols and incubated either one or three hours. Linearity R^2 values of greater than 0.98 are typical for the CyQUANT™ Direct Red and CyQUANT™ Direct Cell Proliferation Assays.

The CyQUANT™ Direct Red Assay can be used to measure cytotoxicity because the background suppressor blocks the staining of dead cells and cells with compromised cell membranes, resulting in the fluorescence staining of healthy cells that can be compared between treated and untreated cultures (Figure 2).

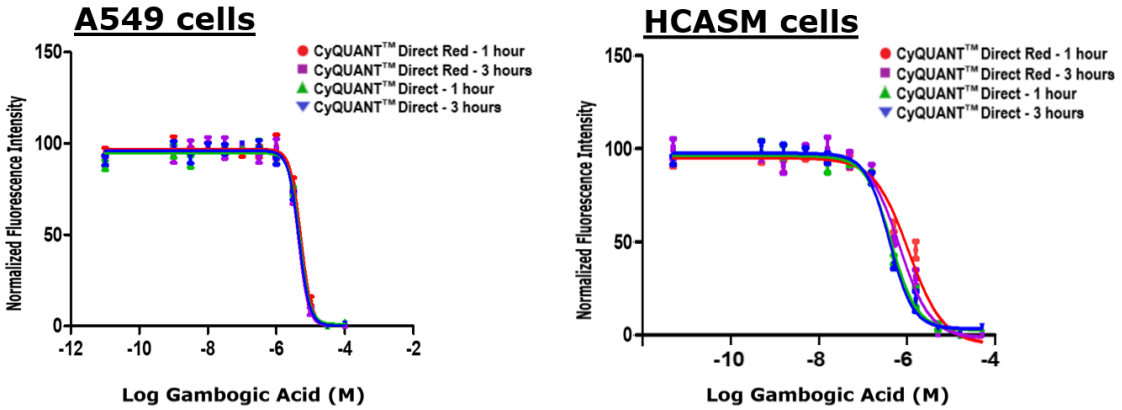

Figure 2 Cytotoxicity measurements using the CyQUANT™ Direct Red Assay

Measurements of cytotoxicity differences across different cell types were performed using the CyQUANT™ Direct Red Assay. A549 and HCASM (primary human coronary artery smooth muscle) cells were added into a 96-well plate and incubated overnight. After incubation the cells were treated with gambogic acid and the amount of cytotoxicity was determined by using either the CyQUANT™ Direct Red or CyQUANT™ Direct Assays. Fluorescence measurements were made after one and three hours of incubation using either assay. The decrease in fluorescence can be correlated to the increase in cytotoxicity cause by the gambogic acid treatment

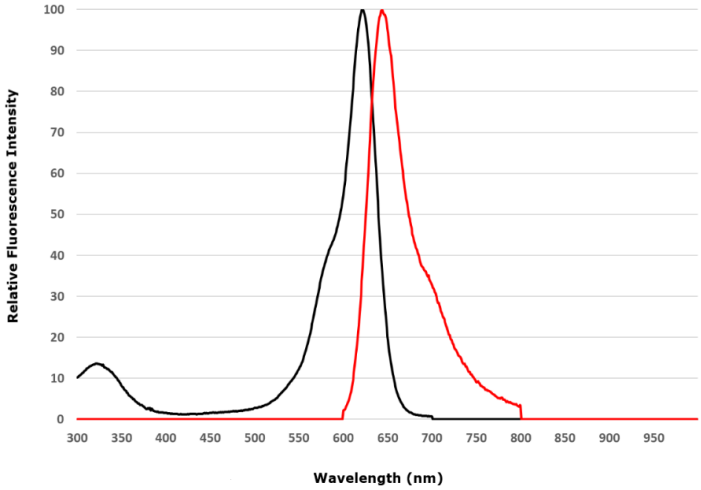

Figure 3 Fluorescence spectra of the CyQUANT™ Direct Red nucleic acid stain

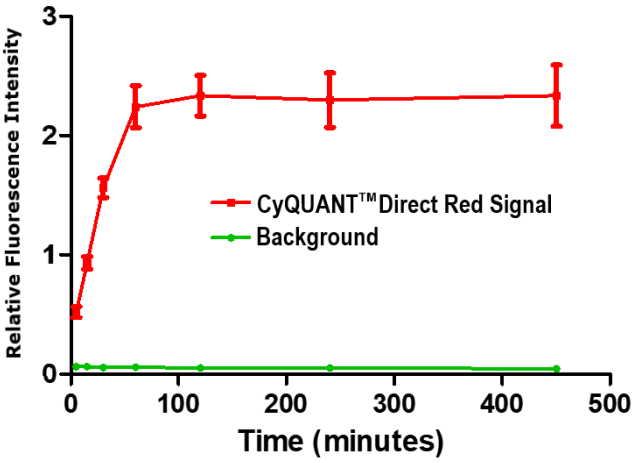

Figure 4 Stability of CyQUANT™ Direct Red Assay Signal

A549 cells were aliquoted and incubated overnight. Using the CyQUANT™ Direct Red assay and following the manufacture's protocol the health cells were labeled in the wells. Fluorescence intensities were measured from 5 minutes to 7.5 hours using a fluorescence microplate reader with a Cy5™/Alexa Fluor 647 excitation/emission filter set. The fluorescence signal intensity reached a plateau within 60 minutes of reagent addition, and remained stable for more than 7 hours, making it extremely work-flow friendly.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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Revision history: Pub. No. MAN0018644 B.0

Revision	Date	Description
B.0	3 May 2023	New combo skus added.
A.0	5 April 2019	New document for CyQUANT Red Assay Kit.