CyQUANT® Direct Cell Proliferation Assay Kit

Catalog nos. C35011, C35012

Table 1. Contents and storage information.

Material	Amount*		Concentration	Storage†	Stability
	10 plate kit (Cat. no. C35011)	100 plate kit (Cat. no. C35012)			
CyQUANT® Direct nucleic acid stain (Component A)	0.5 mL in DMSO	5 mL in DMSO	500X	• 2–25°C • Desiccate • Protect from light	When stored as directed, the product is stable for at least 6 months.
CyQUANT® Direct background suppressor I (Component B)	2.5 mL in ultrapure water	25 mL in ultrapure water	100X		
Peak fluorescence excitation/emission maxima: CyQuant® Direct nucleic acid stain: 508/527 nm, bound to nucleic acids.					
* See Table 2 for number of assays. † For long-term storage (i.e., ≥ 6 months), store components at ≤–20°C.					

Table 2. Number of assays in CyQUANT® Direct Cell Proliferation Assay kits.

	10 plate kit (Cat. no. C35011)			100 plate kit (Cat. no. C35012)		
	96-well plate	384-well plate	1,536-well plate	96-well plate	384-well plate	1,536-well plate
Volume per well	100 µL/well	25 µL/well	5 µL/well	100 µL/well	25 µL/well	5 µL/well
Number of assays	960	3,840	15,360	9,600	38,400	153,600

Introduction

CyQUANT® Direct Cell Proliferation Assay is a fluorescence-based proliferation and cytotoxicity assay for microplate readers. The no-wash, homogenous format and fast add-mix-read protocol makes the CyQUANT® Direct assay ideal for HTS applications. The assay can be completed in one hour, with no washes, cell lysis, temperature equilibrations, or radioactivity required.

The CyQUANT® Direct assay is based on a cell-permeant DNA-binding dye in combination with a background suppression reagent. As DNA content is highly regulated, cell number estimates are very accurate. The masking dye blocks staining of dead cells and cells with compromised cell membranes, causing only staining of healthy cells. Therefore, the CyQUANT® Direct assay measures proliferation as well as cytotoxicity. The concordance of the CyQUANT® Direct assay with cytotoxicity assays based on cell metabolism or energy states is excellent (Figure 1 and Table 3).

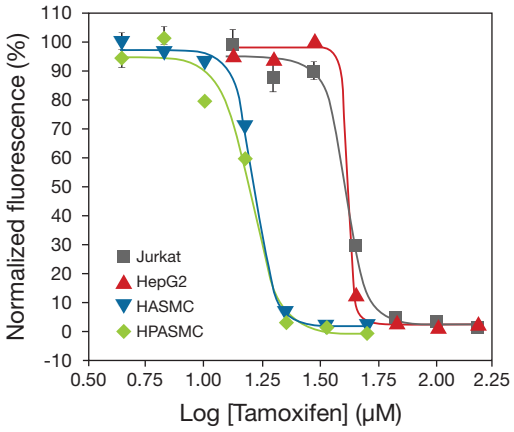

Figure 1. Cytotoxicity measurements using the CyQUANT® Direct assay. Measurements of cytotoxicity differences across different cell types were performed using the CyQUANT® Direct assay. Hep-G2, Jurkat, human aortic smooth muscle cells (HASMC), and human pulmonary aortic smooth muscle cells (HPASMC) were seeded in 384-well plates at a density of 5,000 cells per well in 30 µL medium containing 10% FBS. Following incubation at 37°C for 48 hours with increasing concentrations of Tamoxifen, 30 µL of CyQUANT® Direct reagent was added into each well. Fluorescence measurements were made after 60 minutes. Fluorescence intensities were normalized to DMSO alone treatment, and IC_{50} curves were generated using GraphPad Prism® software. The results shown represent averages of readings from eight wells per data point. As shown in the figure, the two primary cell types (HASMC and HPASMC) were significantly more sensitive to Tamoxifen treatment than two transformed cell lines, adherent Hep-G2, and suspension Jurkat cells.

Table 3. Correlative results of CyQUANT® Direct assay to metabolism-based assays.*

Readout **Cell Type**	**CyQUANT® Direct assay** (DNA, cell membrane integrity)	**CellTiter-Glo® assay** (ATP)	**alamarBlue® assay** (resazurin; red/ox potential)	**CellTiter 96® AQ_{ueous} assay** (MTS; red/ox potential)
Hep-G2	40.7	39.6	44.6	41.9
Jurkat	41.5	35.8	35.5	43.7
HASMC	16.4	13.8	16.8	15.5
HPASMC	15.8	12.3	16.1	15.8

* Assays were performed under the conditions described in Figure 1 according to the manufacturers' recommendations, and in all cases, eight data points were obtained per concentration of Tamoxifen.

With a dynamic range from less than 50 to more than 20,000 cells of most adherent and suspension cell types, the CyQUANT® Direct assay can be used in 96-, 384-, or 1,536-well microplate formats, and is compatible with most HTS and HCS readers. Because the experimental protocol does not include a lysis step, the assay can conveniently be multiplexed using a spectrally distinct fluorescent or a luminescent readout.

The CyQUANT® Direct Cell Proliferation Assay kit consists of two components, a green fluorescent nucleic acid stain (see Figure 2 for fluorescence spectra), and a background suppression dye. The nucleic acid stain is a live cell permeable reagent that mainly concentrates in the nucleus of mammalian cells, whereas the suppression dye is impermeable in live cells and suppresses "green" fluorescence, thus eliminating the need to perform wash steps (Figure 3). The combination of these two components results in an assay based on both DNA content and membrane integrity. 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. Since fluorescence intensity is linearly dependent on cell number, the assay can be made quantitative by using a standard curve of known cell numbers.

The CyQUANT® Direct Cell Proliferation assay is designed for use with adherent and suspension mammalian cells. As cells are not lysed or permeabilized, and because staining does not require active metabolism, the readout provides a direct measure of cell proliferation and viability. The CyQUANT® Direct 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.

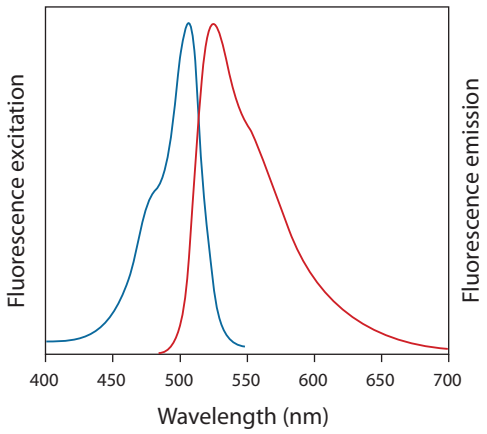

Figure 2. Fluorescence spectra of CyQUANT® Direct nucleic acid stain.

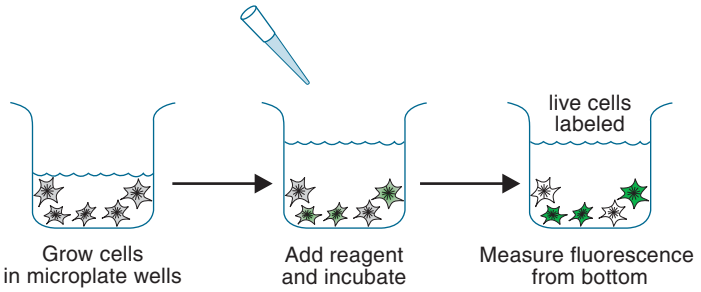

Figure 3. CyQUANT® Direct assay. The CyQuant® Direct assay is a homogenous, non-lytic cell proliferation and cytotoxicity assay designed for use with multi-well plates (96-, 384-, or 1,536-well plate formats), making it ideal for high-throughput screening applications. The reagent is added directly to cells in complete medium, incubated for 30 to 60 minutes, and read on standard plate readers.

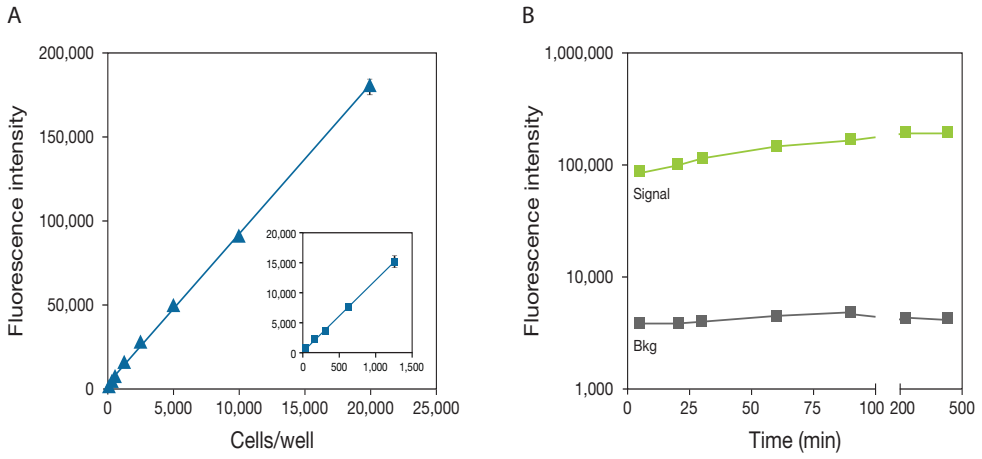

Figure 4. Linearity (panel A) and stability (panel B) of CyQUANT® Direct assay signal. (A) CHO cells (M1WT3 ATCC CRL-1985) were plated at densities of 0–20,000 per well in a 384-well poly-D-lysine-coated microplate. Cells were labeled according to the microtiter plate assay format protocol described in this product information sheet. Fluorescence intensities, measured with a fluorescence microplate reader using FITC filter set, varied linearly with respect to cell number over the range of 40 to 20,000 cells. The inset shows the measurement range from 0–1,250 cells per well. The results shown represent averages of eight experiments per data point. **(B)** CyQUANT® Direct 2X detection reagent was added to adherent CHO cells in culture media with serum, and the fluorescence was read from 5 minutes to 7.5 hours after reagent addition. The fluorescence signal intensity reached a plateau within 30–60 minutes of reagent addition, and remained stable for more than 7 hours, making it extremely work-flow friendly.

With a dynamic range from less than 50 to more than 20,000 cells of most adherent and suspension cell types, the CyQUANT® Direct assay can be used in either 96-, 384-, or 1,536-well microplate formats, and is compatible with most HTS and HCS readers. Because the experimental protocol does not include a lysis step, the assay can conveniently be multiplexed using a spectrally distinct fluorescent or a luminescent readout.

The CyQUANT® Direct Cell Proliferation Assay kit consists of two components: A) a green fluorescent nucleic acid stain (Figure 2), and B) a background suppression dye. The nucleic acid dye is a live cell permeable reagent that mainly concentrates in the nucleus of mammalian cells, whereas the suppression dye is impermeable in live cells and suppresses "green" fluorescence, thus eliminating the need to perform wash steps (Figure 3). The combination of these two components results in an assay based on both DNA content and membrane integrity. 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. Since fluorescence intensity is linearly dependent on cell number, the assay can be made quantitative by using a standard curve of known cell numbers.

The CyQUANT® Direct Cell Proliferation assay is designed for use with mammalian cells of both adherent and suspension types. As cells are not lysed or permeabilized, and because staining does not require active metabolism, the readout provides a direct measure of both cell proliferation and viability. The CyQUANT® Direct 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.

Before You Begin

Caution

CyQUANT® Direct nucleic acid stain (Component A) contains DMSO, which is known to facilitate the entry of organic molecules into tissues. There is no data available addressing the mutagenicity or toxicity of CyQUANT® Direct nucleic acid stain (Component A). Because this reagent binds nucleic acids, treat it as a potential mutagen and handle with appropriate care.

When handling the reagents, wear appropriate gloves, protective clothing, and eyewear, and follow safe laboratory practices. We recommend using impervious butyl rubber gloves when handling the reagents. We do **not** recommend using nitrile gloves as some brands of nitrile gloves have breakthrough times of five minutes.

Dispose of reagents in accordance with local regulations.

Types of Cells

The CyQUANT® Direct assay has been validated on a number of mammalian cell types, including adherent (CHO-K1, Hep-G2, U87, MDA-MB-231, SNU-1, PC-3, human primary aortic smooth muscle cells (HASMC), human primary pulmonary arterial smooth muscle cells (HPASMC), human keratinocytes, and HUVEC) and suspension cells (Jurkat).

Both suspension and adherent cell types are amenable to this assay; however, you must allow suspension cells to settle to the bottom of the well before reading the fluorescence. If necessary, centrifuge the plates gently to aid settling of suspension cells.

The assay can tolerate a variety of cell culture media components including phenol red and up to 10% serum.

Preparing the 2X Detection Reagent

The following recipe is for preparing 12 mL of 2X detection reagent, which is sufficient for one microtiter plate with 100 µL/well in a 96-well plate, 25 µL/well in a 384-well plate, and 5 µL/well in a 1,536-well plate.

1.1 Combine the following in 15 mL tube:

Hank's buffered saline solution, PBS, or cell culture media (with or without serum)	11.7 mL
CyQuant® Direct nucleic acid stain	48 µL
CyQuant® Direct background suppressor I	240 µL

1.2 Mix the 2X detection reagent well and let stand at room temperature until ready for use.

Note: When prepared aseptically, the 2X detection reagent is generally stable for up to 24 hours at room temperature.

Preparing Cells

Prepare the cells according to your experimental needs as follows.

Viability/Proliferation Assay

2.1 Plate cells on a microscope slide or a microtiter plate.

2.2 You may grow the cells for multiple days or assay them immediately.

Cytotoxicity Assay

3.1 Plate cells on a microscope slide or a microtiter plate.

3.2 Add the test compound(s).

3.3 Incubate cells with the test compound(s) for 24–96 hours before commencing the CyQUANT® Direct assay.

Experimental Protocols

Microtiter Plate Assay Format

4.1 Add an equal volume of 2X detection reagent to cells in culture.

For example, add 100 µL of 2X detection reagent to 100 µL of cells in cell culture media or buffer.

4.2 Incubate cells with the detection reagent for 60 minutes at 37°C.

4.3 Read fluorescence of samples using standard "green" filter sets (i.e., FITC filter set) or appropriate wavelengths (i.e., 480/535 nm, if using a monochrometer-based instrument).

Note: A bottom-read plate 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.

Microscope Assay Format

5.1 Add an equal volume of 2X detection reagent to cells in culture.

For example, add 100 µL of 2X detection reagent to 100 µL of cells in cell culture media or buffer.

5.2 Incubate cells with the detection reagent for 60 minutes at 37°C.

5.3 Image sample(s) using standard "green" filter sets (i.e., FITC filter set).

Single Tube Assay Format (Fluorescence Spectrophotometer or Flow Cytometer)

6.1 Add an equal volume of 2X detection reagent lacking CyQUANT® Direct background suppressor I to cells in culture.

For example, add 100 µL of 2X detection reagent (minus background suppressor) to 100 µL of cells in cell culture media or buffer.

6.2 Incubate cells with the detection reagent (minus background suppressor) for 60 minutes at 37°C.

6.3 Remove detection reagent by detaching adherent cells from the dish, and then pelleting cells by centrifugation for 5 minutes at 200 × *g*.

6.4 Resuspend cells in cell culture media, Hank's buffer, or PBS.

6.5 Read the fluorescence of sample using standard "green" filter sets (i.e., FITC filter set).

Note: When using a spectrophotometer, make sure that the cells are in suspension.

***Optional*: Constructing a Standard Curve**

You can create a reference standard curve for converting sample fluorescence values into cell numbers. Use the same cell type used in the experiment for the standard curve. You may assay suspension cells or adherent cells; however, you must first detach and resuspend the adherent cells by trypsin treatment. Note that some adherent cells are sensitive to trypsinization and some cell lysis might ensue.

7.1 Prepare a concentrated cell suspension in medium; ideally, the cells suspension should have ~1 mL total volume at a density of about 10^5–10^6 cells/mL. Determine the actual cell density by counting the cells using a manual hemacytomer or an automated cell counter such as the Countess™ Automated Cell Counter.

7.2 Generate a dilution series in the wells of a microtiter plate. Use cell culture media or PBS, and make dilutions corresponding to cell numbers ranging from 50 to 50,000 cells in 25 µL (384-well plate) or 100 µL (96-well plate) volumes. Include a sample with no cells as a negative control.

7.3 Proceed with detection by following the protocol outlined above for the Microtiter Plate Assay Format.

Frequently Asked Questions:

General Questions

Q: What are the peak excitation and emission wavelengths of the CyQUANT® Direct nucleic acid stain?
A: The peak excitation wavelength is 508 nm and the peak emission wavelength is 527 nm.

Q: What cell types work with the CyQUANT® Direct Cell Proliferation Assay?
A: Adherent or suspension cells including CHO-K1, Jurkat, Hep-G2, U87, MDA-MB-231, SNU-1, PC-3, HASMC, HPASMC, Keratinocytes, and HUVEC.

Q: Is the CyQUANT® Direct Cell Proliferation Assay compatible with flow cytometry and fluorescence spectrophotometer readings?
A: Yes. Follow the Single Tube Assay Format protocol above, which omits CyQUANT® Direct background suppressor I in the 2X detection reagent

Storage Questions

Q: Can I store the CyQUANT® Direct Cell Proliferation Assay Kit at room temperature?
A: Yes, the assay kit is stable at ambient temperature for up to 6 months.

Q: Can I freeze the CyQUANT® Direct Cell Proliferation Assay Kit prior to use?
A: Yes, the assay kit is stable through multiple freeze and thaw cycles.

Q: Can I store the 2X detection reagent for later use once it is made?
A: Yes. Generally speaking, you can store the 2X detection reagent refrigerated for up to one week.

Methods Questions

Q: When working in a microtiter plate, do I need to remove the media or the free dye?
A: No, the CyQUANT® Direct assay was specially formulated with a background suppression dye that eliminates the need for media removal or wash steps.

Q: Can I freeze cell samples from multiple assay time points and assay later at the same time?
A: No, the CyQUANT® Direct assay contains a background suppression reagent that requires an intact plasma membrane to discriminate between live and dead cells. If you are interested in freezing samples prior to performing the assay, consider using the CyQUANT® Cell Proliferation Assay (Cat. no. C7026).

Q: Does phenol red or 10% serum interfere with the assay?
A: No, the CyQUANT® Direct assay is compatible with phenol red and serum from cell culture media.

References

1. J Immunol Meth 142, 199 (1991); **2.** J Immunol Meth 254, 85 (2001); **3.** J Mol Neurosci 21, 895 (2003); **4.** Toxicol in vitro 18, 639 (2004); **5.** Nucleic Acids Res 32, 6585 (2004); **6.** Toxicol in vitro 20, 785 (2005); **7.** ASSAY Drug Dev Technol 4, 555 (2006); **8.** J Biomol Screening 13, 527 (2008).

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
C35011	CyQUANT® Direct Cell Proliferation Assay *for 10 microplates*	1 kit
C35012	CyQUANT® Direct Cell Proliferation Assay *for 100 microplates*	1 kit
Related products		
C7026	CyQUANT® Cell Proliferation Assay Kit *for cells in culture* *1000 assays*	1 kit
C7027	CyQUANT® cell-lysis buffer *20X concentrate*	50 mL
C35006	CyQUANT® NF Cell Proliferation Assay Kit *1000 assays*	1 kit
C35007	CyQUANT® NF Cell Proliferation Assay Kit *200 assays*	1 kit
C10227	Countess™ Automated Cell Counter	1 each

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Toll-Free Ordering for USA:
Order Phone: (800) 438-2209
Order Fax: (800) 438-0228

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