An absorbance-based assay for cell health and proliferation

CyQUANT XTT Cell Viability Assay.

Measuring changes in cell viability is fundamental when assessing cell health, determining gene toxicity, and evaluating anticancer drugs. Many assays are available for determining different parameters of cell health such as metabolism, membrane integrity, and enzyme and channel activity. These assays can be used to provide a snapshot of cell viability or, if used on sequential days, to monitor cell proliferation. Choosing the viability assay that best addresses your research question requires a closer look at the differences between these assays, including the mechanistic readout of cell health status, the detection methods, and the assay sensitivity.

The fastest and easiest way to determine cell health is with an add-and-read (continuous) assay designed for detection with a microplate reader. Ideal for high-throughput applications, the Thermo Scientific™ Varioskan™ LUX Multimode Microplate Reader paired with Thermo Scientific™ SkanIt™ Software allows for fast readout of absorbance and fluorescence signals, and instantaneous access to data processing steps such as curve fitting, cell viability calculations, and cytotoxicity potencies.

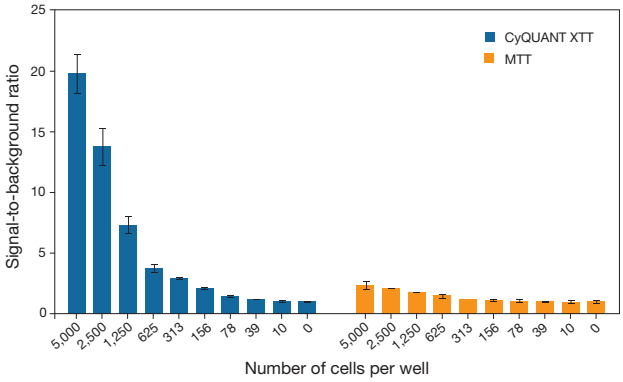

Figure 1. Comparison of the signal-to-background ratios provided by XTT- and MTT-based viability assays. A549 cells were assayed using either the Invitrogen™ CyQUANT™ XTT Cell Viability Assay (Cat. No. X12223) or a commercially available MTT assay according to their respective manufacturers' protocols. The signal-to-background ratios provided by the CyQUANT XTT assay were much greater than those produced with the MTT assay. See Figure 2 for a definition of signal-to-background ratio for the XTT assay product. The signal-to-background ratio for the MTT assay product is calculated as the absorbance of the sample at 540 nm divided by the absorbance of the blank at 540 nm. All measurements were made using a Thermo Scientific™ Varioskan™ LUX Multimode Microplate Reader.

Assay cell viability with tetrazolium salts

Metabolically active cells can oxidize or reduce a variety of chromogenic or fluorogenic probes, providing a measure of cell viability and overall cell health. Tetrazolium salts are widely used for detecting the redox potential of cells for absorbance-based viability, cytotoxicity, and proliferation assays. Following reduction, these water-soluble, colorless compounds are converted to uncharged, brightly colored (nonfluorescent) formazans. Several of the formazans precipitate out of solution and are useful for histochemical localization of the site of reduction or, after solubilization in organic solvent, for quantitation by standard spectrophotometric techniques.

The reduction of MTT remains the most common assay for tetrazolium salt–based viability testing. However, the typical MTT assay requires more than 8 hours to perform, including two separate 4-hour incubation steps. Additionally, because the purple-colored formazan product formed from the reduction of MTT is insoluble, treatment with an acid or DMSO is required to solubilize the formazan before acquiring the data on a microplate reader. Unlike MTT's formazan product, the extremely water-soluble formazan product of XTT does not require solubilization prior to quantitation, thereby reducing the assay time in most viability assay protocols. Moreover, the sensitivity of the XTT reduction assay is reported to be similar to or better than that of the MTT reduction assay [1].

The Invitrogen™ CyQUANT™ XTT Cell Viability Assay provides the XTT reagent for assessing cell viability as a function of cellular redox potential. In the presence of actively respiring cells, XTT is converted to a water-soluble, orange-colored formazan product. In addition, because the CyQUANT XTT assay does not require cell lysis and uses a noninvasive probe, stained cells can be further analyzed with other cell function probes. Compared with an MTT-based assay, the CyQUANT XTT assay displays up to an 8-fold increase in signal-to-background ratios, offering significant advantages when high sensitivity is required for detection of small cell populations or poorly metabolizing cells (Figure 1). This assay can be completed in as little as 2 hours, though 4 hours is recommended for optimal results.

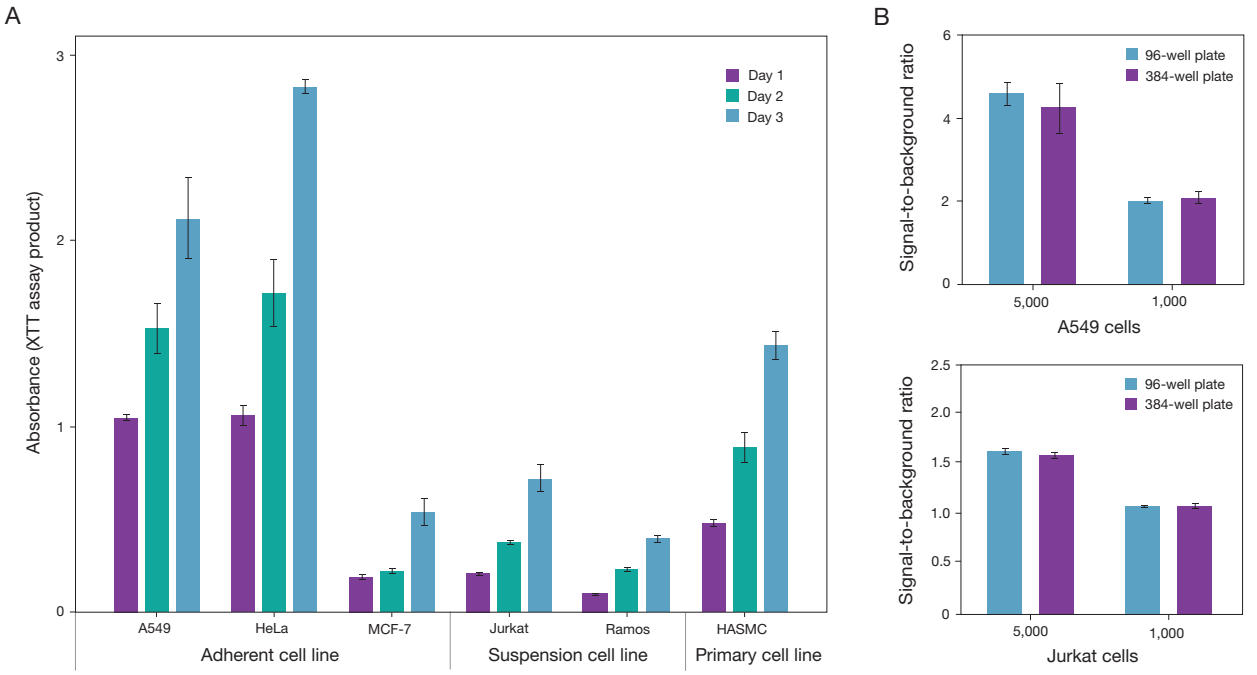

Figure 2. Versatility of the CyQUANT XTT assay. (A) The Invitrogen™ CyQUANT™ XTT Cell Viability Assay (Cat. No. X12223) was used with a variety of cell types, including adherent cells (A549, HeLa, and MCF-7 cells), suspension cells (Jurkat and Ramos cells), and primary cells (human aortic smooth muscle cells, HASMCs), to evaluate cell viability and proliferation. Each cell line was seeded onto three independent plates on day 1, and baseline viability was measured using the CyQUANT XTT assay. The assay was repeated on days 2 and 3, and the absorbance increase over the 3-day period is indicative of cell proliferation. **(B)** The CyQUANT XTT Cell Viability Assay produced nearly identical results when used to evaluate viability of A549 and Jurkat cells in 96- and 384-well assay plates and at two different cell densities. Absorbance of the XTT assay product is reported as the absorbance of the sample at 450 nm minus the absorbance of the sample at 660 nm (this subtracted value is the signal) minus the absorbance of the blank at 450 nm. The signal-to-background ratio is calculated as the signal divided by the absorbance of the blank at 450 nm. All measurements were made using a Thermo Scientific™ Varioskan™ LUX Multimode Microplate Reader.

Simplify viability measurements with the CyQUANT XTT assay

The CyQUANT XTT Cell Viability Assay is a complete and easy-to-use kit for the detection of mammalian cell viability. This kit includes the XTT reagent (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide) and an Electron Coupling Reagent, each provided in single-use vials; sufficient reagents are provided for ten 96-well plates, and the kit is stored at –20ºC. The reduction of XTT is facilitated by incorporation of the Electron Coupling Reagent, an electron mediator that scavenges readily available electrons on the plasma membrane, leading to the formation of a reactive intermediate that then reduces XTT to its intensely colored formazan product.

The CyQUANT XTT viability assay protocol is simple: once cells are seeded and treated with the metabolite or drug of interest, the XTT reagent and the Electron Coupling Reagent are thawed, mixed together, and added to cells. After the cells are incubated at 37ºC, their absorbance is measured at 450 nm (the absorption maximum of the orange XTT reduction product) and at 660 nm (to measure background signals due to cell debris and excess coupling reagent) using an absorbance-based plate reader such as the Varioskan LUX Multimode Microplate Reader.

The CyQUANT XTT Cell Viability Assay has been developed to work across a wide range of cell lines, including adherent, suspension, and primary cell types (Figure 2A), and with both 96- and 384-well plates (Figure 2B). The sensitivity and dynamic range of the CyQUANT XTT assay is significantly increased by the inclusion of the Electron Coupling Reagent. In the protocol, we recommend that the XTT/Electron Coupling Reagent stock solution be used soon after thawing and mixing; assay performance is compromised if this stock solution is kept at room temperature for extended periods of time or subjected to freeze/thaw cycles. To help ensure stability, these reagents are provided in single-use vials and stored at –20ºC until thawed for use. →

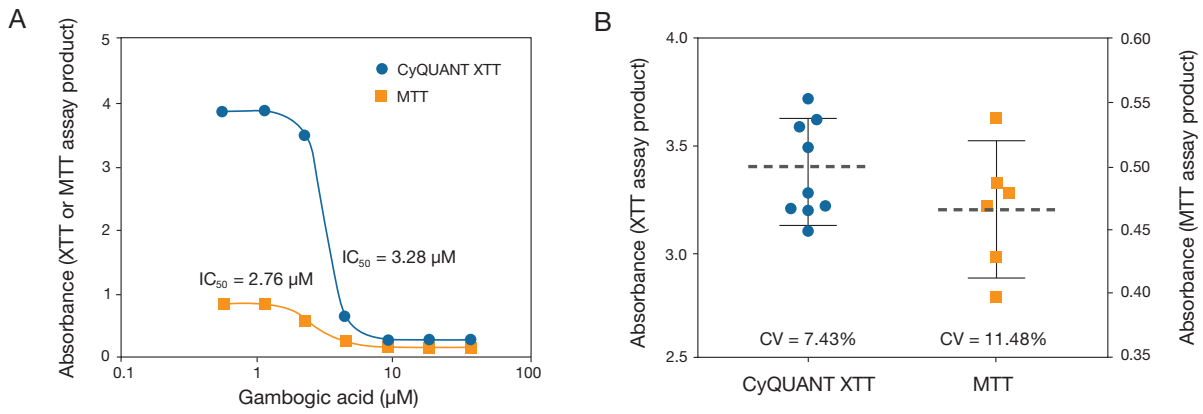

Figure 3. Comparison of CyQUANT XTT and MTT assay sensitivity and variability. (A) The Invitrogen™ CyQUANT™ XTT Cell Viability Assay (Cat. No. X12223) and a commercially available MTT assay were used according to their respective manufacturers' protocols to generate drug dose response curves for A549 cells exposed to increasing concentrations of gambogic acid for ~18 hr at 37°C and 5% CO_2. The IC_{50} value for gambogic acid generated with the CyQUANT XTT assay (3.28 µm) was similar to that obtained with the MTT assay (2.76 µm). **(B)** Variability of the assays was compared by measuring the reduction of either CyQUANT XTT or MTT in multiple wells of untreated cells. The data shown represent an example in which the mean of the assay absorbance (dashed lines) is plotted along with the absorbance of the individual wells. The CV of the CyQUANT XTT assay was smaller than that of the MTT assay. Absorbance of the XTT assay product is reported as the absorbance of the sample at 450 nm minus the absorbance of the sample at 660 nm minus the absorbance of the blank at 450 nm. Absorbance of the MTT assay product is reported as the absorbance of the sample at 540 nm minus the absorbance of the blank at 540 nm. All measurements were made using a Thermo Scientific™ Varioskan™ LUX Multimode Microplate Reader, and data were analyzed using GraphPad Prism software.

Generate drug dose response curves with the CyQUANT XTT assay

We used the CyQUANT XTT Cell Viability Assay to generate drug dose response curves for A549 cells exposed to gambogic acid, a xanthanoid compound extracted from the bark of an evergreen tree native to Southeast Asia. Gambogic acid has been shown to inhibit cell growth through the induction of apoptosis and cell death.

Figure 3A shows a comparison of the drug dose response curves obtained with the CyQUANT XTT assay and the MTT assay. The CyQUANT XTT Cell Viability Assay has an IC_{50} for gambogic acid of approximately 3 µM, similar to that of the MTT assay. Furthermore, this study shows that the CyQUANT XTT assay measures cell viability with greater sensitivity (Figure 3A) and less variability (Figure 3B) than does the MTT assay.

Add the XTT assay to your lab's repertoire

The CyQUANT XTT Cell Viability Assay is a complete, optimized, easy-to-use kit for sensitive colorimetric detection of viable mammalian cells using a microplate reader. Unlike other commercially available viability assays, the CyQUANT XTT assay displays a large dynamic range and low well-to-well variability and can be multiplexed with other cell function probes. For more information on absorbance- or fluorescence-based microplate assays for viability, visit **thermofisher.com/microplate-viability**. ■

Reference

1. Meshulam T, Levitz SM, Christin L et al. (1995) *J Infect Dis* 172:1153–1156.

Product	Quantity	Cat. No.
CyQUANT™ XTT Cell Viability Assay	1 kit	X12223
Varioskan™ LUX Multimode Microplate Reader, absorbance and fluorescence intensity measurements, top-reading	1 each	VL0000D0 VL0000D1
Varioskan™ LUX Multimode Microplate Reader, absorbance and fluorescence intensity measurements, top- and bottom-reading	1 each	VLB000D0
Varioskan™ LUX Multimode Microplate Reader, absorbance, fluorescence intensity, and luminescence measurements, top-reading	1 each	VL0L00D0
Varioskan™ LUX Multimode Microplate Reader, absorbance, fluorescence intensity, and luminescence measurements, top- and bottom-reading	1 each	VLBL00D0
SkanIt™ Software for Microplate Readers, Research Edition	1 each	5187139