CyQUANT™ XTT Cell Viability Assay

Catalog No. X12223

Pub. No. MAN0017857 **Rev.** A.0

Product information

The CyQUANT™ XTT Cell Viability Assay is a complete and optimized kit for the detection of mammalian cell viability. The assay kit includes the XTT reagent (2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2*H*-Tetrazolium-5-Carboxanilide) and an Electron Coupling Reagent. The XTT reagent, which is a tetrazolium-based compound, is sensitive to cellular redox potential. Actively respiring cells convert the water-soluble XTT compound to an orange colored formazan product. The sensitivity and consistency of the assay is significantly increased when used with the Electron Coupling Reagent.

For each 96-well plate, one bottle of XTT and one vial of the Electron Coupling reagent are thawed, mixed to create a working solution, then added to the cells. After 2–4 hours of incubation, cell viability is detected by measuring absorbance at 450 nm and 660 nm. The assay kit supplies all the materials needed for the detection of mammalian cell viability in ten 96-well plates.

Table 1. Contents and storage

Product	Amount	Storage*
XTT Reagent (Component A)	10 bottles (6 mL/bottle)	• Store at ≤ –20°C • Protect from light
Electron Coupling Reagent (Component B)	10 vials (1 mL/vial)	
* When stored as directed, kit components are stable for 6 months from the date of receipt.		
Number of assays: Sufficient material is supplied for 10 × 96-well plates, based on the protocol described here.		
Approximate absorbance maximum: 450 nm		

Advantages of the CyQUANT™ XTT assay

Measuring changes to cell viability is a fundamental method for assessing cell health, determining genotoxicity, and evaluating anti-cancer drugs. In addition to being cost effective, colorimetric-based viability assays generate consistent results with low well-to-well variability. Unlike other commercially available colorimetric-based mammalian cell viability assays, XTT-based assays have been demonstrated to display a high dynamic range and low variability.

Since no cell lysis or solubilization are required for detection, the XTT assay can be used as a continuous assay, allowing several time points to be collected during the course of the experiment. Additionally, the CyQUANT™ XTT assay has been demonstrated to generate viability results from a wide range of cell lines, including primary and suspension cells.

Methods

Important procedural guidelines

- Use the XTT/Electron Coupling Reagent working solution soon after mixing. Assay performance will be compromised and sensitivity reduced if the stock solution is kept at room temperature for extended periods of time or subjected to freeze/thaw cycles.

  To ensure assay performance and reproducibility the CyQUANT™ XTT Cell Viability assay consists of separate XTT and Electron Coupling Reagents, which are mixed immediately before use.

- Background absorbance signal can be caused by various factors such as cell culture conditions, type of media used, or exposure to light. To correct for the background absorbance, include at least three wells that only contain complete culture medium without cells. These wells will be used as the **Blank** wells, and the wells containing cells will be the **Test** wells.

  When determining the specific absorbance of the sample, the absorbance readings from the **Blank** wells will be used to correct the signal from the **Test** wells for background.

Seed cells

1.1 One day before your experiment, seed cells into a 96-well plate containing 100 µL/well of cell culture medium. Make sure to include at least three wells that contain only complete culture medium without the cells as your **Blank** wells.

Note: If you are using a 384-well plate, use 50 µL/well of cell culture medium.

1.2 (*Optional*) To correlate the specific absorbance signal to the number of viable cells, create a standard curve from a dilution of cells. We recommend that you use a range of 10^3–10^5 cells/well to generate this standard curve. Leave these wells untreated, but add the XTT working solution.

1.3 Incubate the cells overnight in a 37°C incubator.

Prepare working solution

2.1 On the day of the experiment, remove one package containing one bottle of XTT Reagent and one vial of the Electron Coupling Reagent from the CyQUANT™ XTT Cell Viability Assay kit stored at –20°C.

2.2 Thaw the XTT Reagent (Component A) in a 37°C water bath. Vortex the bottle to ensure that the XTT Reagent is fully in solution.

2.3 Thaw the Electron Coupling Reagent at room temperature. Vortex the vial to ensure that the reagent is fully in solution.

2.4 To prepare the working solution, remove 6 mL of XTT Reagent and place it into a 15-mL tube.

2.5 Add 1 mL of the Electron Coupling Reagent to the XTT Reagent in the 15-mL tube, then mix the working solution by vortexing.

IMPORTANT! Use the working solution immediately after preparation.

Label cells and determine specific absorbance

The following protocol can be used for both adherent and suspension cells.

3.1 Immediately after preparation, add 70 µL of the working solution directly to each **Test** well of the 96-well plate (i.e., wells containing cells in 100 µL of cell culture medium).

3.2 Add 70 µL of the working solution directly to each **Blank** well of the 96-well plate (i.e., wells containing only 100 µL of cell culture medium).

Note: If you are using a 384-well plate, add 35 µL of the working solution directly to each **Test** and **Blank** well.

3.3 Incubate the cells at 37°C for 4 hours.

3.4 After incubation, read the absorbance at 450 nm and 660 nm.

Note: The XTT-specific absorbance is measured at 450 nm. The 660 nm absorbance reading is used to eliminate the background signal contributed by cell debris or other non-specific absorbance.

3.5 Determine the specific absorbance of the sample using the following formula:

$$\text{Specific Absorbance} = [\text{Abs}_{450\ \text{nm}}(\textbf{Test}) - \text{Abs}_{450\ \text{nm}}(\textbf{Blank})] - \text{Abs}_{660\ \text{nm}}(\textbf{Test})$$

3.6 Compare the specific absorbance signal from the treated cells to the signal from the dilution series or untreated cells to determine the viable cells per well and how they react to the treatment.

Ordering information

Cat. No.	Product name	Unit size
X12223	CyQUANT™ XTT Cell Proliferation Assay	1 kit

Documentation and support

Customer and Technical Support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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 found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **thermofisher.com/support**.

The information in this guide is subject to change without notice.

For Research Use Only. Not for use in diagnostic procedures.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0017857

Revision	Date	Description
A.0	11 May 2018	New user guide

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Manufacturer: Life Technologies Corporation | 29851 Willow Creek Road | Eugene, OR 97402

Trademarks: All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.

ThermoFisher SCIENTIFIC