



## XTT Cell Proliferation Assay Kit

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Item No. 10010200

[www.caymanchem.com](http://www.caymanchem.com)

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## GENERAL INFORMATION

### Materials Supplied

Kit will arrive packaged as a -20°C kit. For best results, remove components and store as stated below.

Item Number	Item	96 Well Quantity/Size	480 Well Quantity/Size	Storage
600488	XTT Developer Reagent	1 vial/600 µl	5 vials/600 µl	-20°C
10010354	Electron Mediator Solution	1 vial/600 µl	5 vials/600 µl	-20°C

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.



**WARNING:** THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

### Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user **must** review the **complete** Safety Data Sheet, which has been sent *via* email to your institution.

### Precautions

Please read these instructions carefully before beginning this assay.

## If You Have Problems

### Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

## INTRODUCTION

### About This Assay

Cayman's XTT cell proliferation assay provides a tool for studying induction and inhibition of cell proliferation in any *in vitro* model. The assay is based on the extracellular reduction of XTT by NADH produced in the mitochondria via *trans*-plasma membrane electron transport and an electron mediator.<sup>1</sup> XTT produces a water-soluble formazan which dissolves directly into the culture medium, eliminating the need for an additional solubilization step. The XTT Cell Proliferation Assay is preferred over other tetrazolium salt-based assays due to superior performance at extremely low cell densities. XTT can detect as few as 500 cells in a well.

## PRE-ASSAY PREPARATION

### Reagent Preparation

#### XTT Mixture

Immediately before use, thaw the Electron Mediator Solution (Item No. 10010354) and XTT Developer Reagent (Item No. 600488). Combine equal volumes of Electron Mediator Solution with XTT Developer Reagent to make enough XTT Mixture for the number of wells in your experiment and mix well.

If the entire volume will not be used in a single experiment, we recommend that you aliquot and store it at -20°C. When stored at -20°C, the XTT Mixture will be stable for several months. Avoid repeated freeze/thaw cycles.

## ASSAY PROTOCOL

### Procedure

1. Seed cells in a 96-well plate at a density of  $10^3$ - $10^5$  cells/well in 100  $\mu$ l of culture medium with or without compounds to be tested. Culture the cells in a CO<sub>2</sub> incubator at 37°C for 24-48 hours.
2. Add 10  $\mu$ l of the prepared XTT Mixture to each well using a repeating pipettor.
3. Mix gently for one minute on an orbital shaker.
4. Incubate the cells for two hours (adherent culture) to four hours (suspension culture) at 37°C in a CO<sub>2</sub> incubator.
5. Before reading the plate, it is important to mix gently on an orbital shaker for one minute to ensure homogeneous distribution of color.
6. Measure the absorbance of each sample using a microplate reader at a wavelength of 450 nm.

## Sample Data

An example of typical data obtained with this assay is shown in Figure 1 (below). Your data will vary depending on the cell line and culture conditions used.

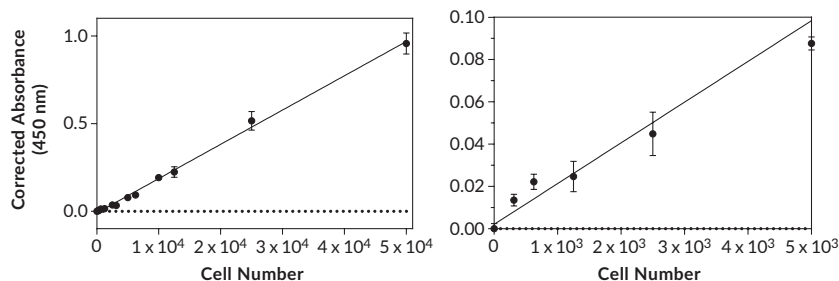


Figure 1: A typical cell titration experiment using HL-60 target cells.

## Reference

1. Berridge, M.V., Tan, A.S., and Herst, P.M. Tetrazolium dyes as tools in cell biology: New insights into their cellular reduction. *Biotechnology Annual Review* **11**, 127-152 (2005).

## NOTES

## Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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