XTT Assay

Catalog number: 22774, 22775
Unit size: 1000 Tests, 5000 Tests

Component	Storage	Amount (Cat No. 22774)	Amount (Cat No. 22775)
XTT Solution	Freeze (< -15 °C)	1000 Tests	5000 Tests

OVERVIEW

The XTT assay is a fast and reliable method for measuring cell viability and proliferation. Based on the reduction of the XTT tetrazolium compound by metabolically active cells, this kit produces a soluble formazan product that can be quantified through absorbance at 450 nm. The assay eliminates the need for washing or solubilization, offering a streamlined workflow and reducing hands-on time.

This XTT assay kit comes in a ready-to-use format making it ideal for high-throughput screening, making it perfect for evaluating cell proliferation in response to various stimuli, such as growth factors, cytokines, and mitogens, as well as assessing cytotoxicity for compounds like anticancer drugs and pharmaceuticals. With its ready-to-use format, this XTT assay is designed to deliver consistent, reproducible results for researchers and labs involved in cell-based assays.

AT A GLANCE

1. Prepare cells in a 96-well plate (100 µL/well).
2. Add 50 µL of XTT Solution to each well.
3. Incubate at 37°C for 1 - 4 hours.
4. Monitor absorbance at OD = 450 nm.

KEY PARAMETERS

Absorbance microplate reader

Absorbance	450 nm and 660 nm
Recommended plate	Clear bottom

CELL PREPARATION

For guidelines on cell sample preparation, please visit: https://www.aatbio.com/resources/guides/cell-samplepreparation.html

SAMPLE EXPERIMENTAL PROTOCOL

Cell Proliferation and Cytotoxicity Assay:

1. Plate 5000 to 10,000 cells/well in a tissue culture microplate with clear bottom.
2. Add test compounds into the cells and incubate for a desired period of time (such as 24, 48, or 96 hours) in a 37°C, 5% CO2 incubator. For blank wells (medium without the cells), add the same amount of test compounds. The suggested total volume is 100 µL for a 96-well plate or 50 µL for a 384-well plate.
   Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density for proliferation or cytotoxicity induction. For proliferation assays, use fewer cells; for cytotoxicity assays, use more cells to start with.
3. Add 50 µL/well (96-well plate) or 25 µL/well (384-well plate) of XTT solution to each well.
4. Incubate the plate at 37°C for 2-5 hours, protect from light.
   Note: The incubation time could be from 30 minutes to overnight depending on the individual cell type and cell concentration used. Optimize the incubation time for each experiment
5. Monitor the absorbance increase with an absorbance plate reader at OD = 450 nm and 660 nm.
   Note: The 660 nm absorbance reading is used to eliminate the background signal contributed by cell debris or other non-specific absorbance.
6. Determine the specific absorbance of the sample using following formula:
   [Abs450 nm(Test) – Abs450 nm(Blank)] – Abs660 nm(Test).

Cell Counting Assay:

1. Prepare cell culture in a tissue culture microplate with a clear bottom. The suggested total volume is 100 µL for a 96-well plate or 50 µL for a 384-well plate.
   Note: We used serially diluted HeLa and Jurkat cell suspension in a clear bottom 96-well plate for the assay.
2. Add 50 µL/well (96-well plate) or 25 µL/well (384-well plate) of XTT Solution to each well.
3. Incubate the plate at 37°C for 2-5 hours, protect from light.
   Note: The incubation time could be from 30 minutes to overnight depending on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.
4. Monitor the absorbance increase with an absorbance plate reader at OD = 450 nm and 660 nm.
   Note: The 660 nm absorbance reading is used to eliminate the background signal contributed by cell debris or other non-specific absorbance.
5. Determine the specific absorbance of the sample using following formula:
   Specific Absorbance = [Abs450 nm(Test) – Abs450 nm(Blank)] – Abs660 nm(Test).

EXAMPLE DATA ANALYSIS AND FIGURES

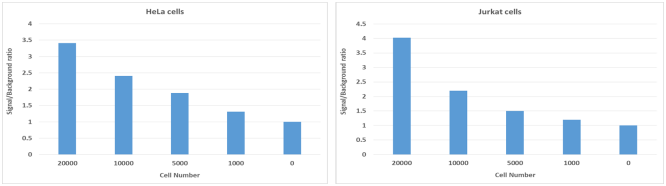

Figure 1. Cell number was determined with XTT Assay. HeLa cells at 1000 to 20,000 cells/well/100 µL were added in a clear bottom 96-well plate. The absorbance was measured at 450 nm and 660 nm at 4 hours after XTT solution incubation with a ClarioStar absorbance microplate reader. The specific absorbance signals were determined using the equation shown on the protocol and signal/background ratio (well with no cells) was measured and plotted.

DISCLAIMER

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