Cell Meter™ Colorimetric MTT Cell Proliferation Kit

Catalog number: 22768, 22769
Unit size: 1000 Tests, 5000 Tests

Component	Storage	Amount (Cat No. 22768)	Amount (Cat No. 22769)
Component A: MTT Reagent A	Freeze (< -15 °C), Minimize light exposure	40 mL	2 X 100 mL
Component B: MTT Reagent B	Room temperature (10-25 °C)	100 mL	500 mL

OVERVIEW

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is a yellow tetrazolium salt commonly used to measure cell viability, proliferation, and cytotoxicity. In live metabolically active cells, water-soluble MTT is reduced in the mitochondria by succinate dehydrogenase to insoluble purple formazan crystals. The amount of formazan produced can be quantified spectrophotometrically (550 nm) upon solubilization and is directly proportional to the number of viable cells in the culture. The MTT assay is a safe, sensitive, and reliable indicator of cell viability and is preferred over other endpoint viability assays, such as the radioactive 3H-thymidine incorporation assay. Besides having a lengthy and tedious protocol, the health hazards of working with radioactive materials are well recognized, as are the accompanying economic considerations required for the appropriate handling and disposal of radioactive waste. [5]

AT A GLANCE

Protocol summary

1. Prepare cells in a 96-well plate (100 µL/well)
2. Add MTT working solution (140 µL/well)
3. Incubate at 37 °C for 2-4 hours
4. Read absorbance at 560 nm

Important Note

Thaw all the kit components at room temperature before use.

KEY PARAMETERS

Absorbance microplate reader

Absorbance	562 nm
Recommended plate	Clear bottom

PREPARATION OF WORKING SOLUTION

MTT working solution

Prepare the amount of MTT working solution needed by mixing 4 mL of MTT Reagent A (Component A) with 10 mL of MTT Reagent B (Component B) (2:5, v/v ratio of MTT Reagent A: B). Mix well.

Note: 14 mL MTT working solution is enough for 100 tests in a 96-well plate. Prepare enough MTT working solution right before the experiment, use promptly.

SAMPLE EXPERIMENTAL PROTOCOL

1. Plate 1000 to 40,000 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, and 25 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 140 µL/well (96-well plate) or 35 µL/well (384-well plate) of MTT working solution to each well.

4. Incubate the plate at 37 °C for 2-4 hours, protect from light.

   Note: The incubation time could be from 2 hours 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 = 562 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Abs@560nm) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Cell Number samples. We recommend using the Online Four Parameter Logistics Calculator which can be found at:

https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-onlinecalculator

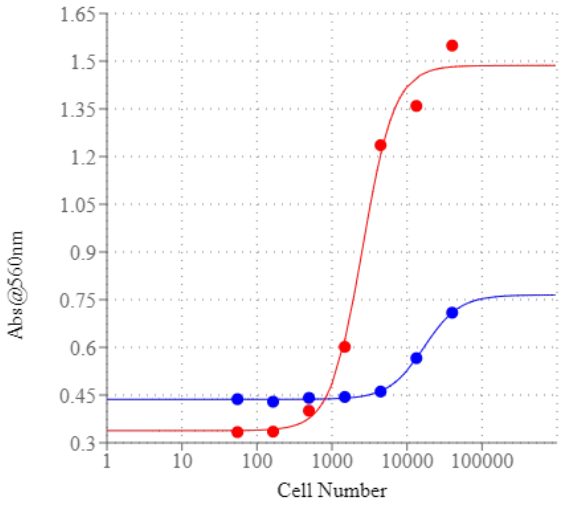


Figure 1. Cell numbers were determined with Cell Meter™ Colorimetric MTT Cell proliferation Kit. HeLa cells at 0 to 40,000 cells/well/100 µL were added in a clear bottom 96-well plate for overnight. The absorbance was measured at 560 nm using a SpectraMax reader (Molecular Devices). 500 cells/well was detected compare to ~5,000 cells/well with Sigma's MTT assay kit.

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