Chondrex, Inc.

MTT Cell Proliferation and Viability Assay Kit

Catalog # 6034

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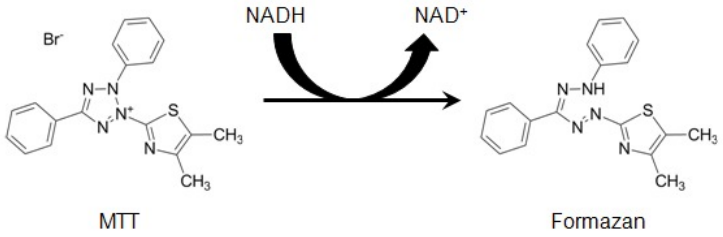
INTRODUCTION

Cell proliferation and viability assays are important, routine techniques in cell biology. These assays are used to evaluate biologically active compounds and drugs and cytotoxic agents, as the assay results correlate with indicators of cellular health, such as intactness of cell membrane and metabolism.

Although there are several assay methods to evaluate cell viability using metabolic activities (ATP or substrates) or DNA synthesis (Thymidine or BrdU incorporation), here, we introduce a tetrazolium reduction assay to evaluate cellular metabolic activity. Several tetrazolium compounds are available as substrates such as MTT, MTS, XTT, and WST-1. Among these compounds, only MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is a positively charged compound which can penetrate viable eukaryotic cells and reflect cytosolic metabolic activities. Soluble MTT is taken into cells, and is reduced by NADH or other reducing molecules by electron transfer. The reduced MTT forms insoluble formazan which accumulates both inside of and on the surface of cells. Generation of formazan reflects viable cell density because dead cells lack the metabolic activity to turn MTT into formazan (1). The resulting formazan can be solubilized and quantified to reflect cell proliferation and viability.



Chondrex, Inc. provides an MTT cell proliferation assay kit (Catalog # 6034) which employs a simple method to assay cell viability and cell densitity using an absorbance microplate reader. This kit can be used for up to 250 samples in duplicate.

KIT COMPONENTS

Item	Quantity	Amount	Storage
MTT Solution (60341)	1 bottle	5 ml	Room Temperature
Solubilizing Solution (60342)	1 bottle	50 ml	Room Temperature

ASSAY PROCEDURES

Standards and samples should be run in duplicate

1. Prepare the cell suspension in culture media at 100 µl/well in 96-well plates.

   Note: 1 x 10^3 to 10^5 cells per well may be appropriate. However, optimization is required.

2. Incubate cells with test reagents for desired period of exposure.

3. Add 10 µl MTT Solution per well.

4. Incubate for 2 - 4 hours at 37°C.

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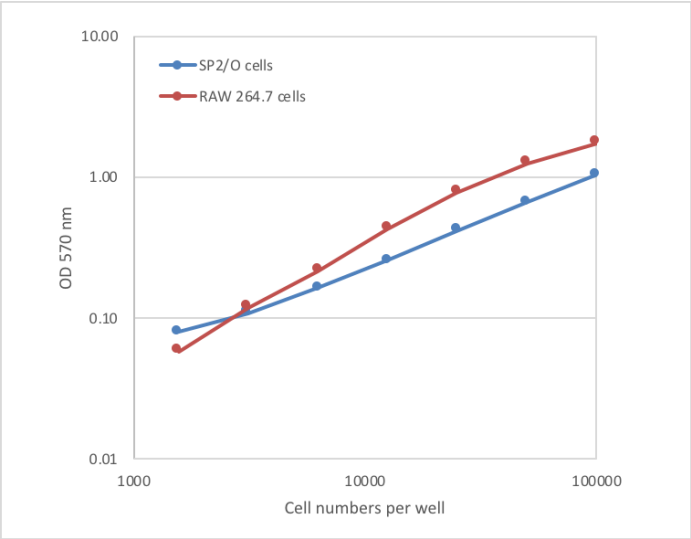
5. Add 100 µl of Solubilizing Solution to each well.
6. Incubate the plate in a dark place at room temperature for 4 hours or overnight.
7. Read absorbance at 570 nm.

CALCULATION OF CELL NUMBERS

1. Average the duplicate OD values for all wells
2. Subtract the averaged OD values of the media-only wells from the averaged OD values of all of the test wells.
3. Compare the OD values between the control group and test group.
   Note: OD values depend on cell type, cell density, and biological activity.

Figure 1 - A typical correlation between OD values and cell numbers in wells



Protocol:

1. Seed cell suspension in culture media at 100 µl/well in 96-well plates.
2. Incubate for 2 hours at 37°C.
3. Add 10 µl of MTT Solution to each well.
4. Incubate for 2 hours at 37°C.
5. Add 100 µl of Solubilizing Solution to each well.
6. Incubate the plate in a dark place at room temperature for 4 hours.
7. Read absorbance at 570 nm.
8. Subtract the averaged OD values of the media-only wells from the averaged OD values of the other wells.
9. Plot OD values against the seeded cell numbers.

REFERENCE

1. P. R. Twentyman, M. Luscombe, A study of some variables in a tetrazolium dye (MTT) based assay for cell growth and chemosensitivity. *Br J Cancer*. **56**, 279–285 (1987).

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