MTT Cell Proliferation and Viability Assay Kit

Catalog # 6034

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	Assay kit to evaluate cell viability and cell density
FORMAT:	96-well ELISA Plate with non-removeable strips
ASSAY TYPE:	Colorimetric Assay
ASSAY TIME:	6+ hours
STANDARD RANGE:	1,000 to 100,000 cells/well
NUMBER OF SAMPLES:	Up to 250 (duplicate) samples/kit
SAMPLE TYPES:	Cultured Cells
RECOMMENDED SAMPLE DILUTIONS:	N/A
CHROMOGEN:	N/A (read at 570 nm)
STORAGE:	-20°C
VALIDATION DATA:	N/A
NOTES:	This kit has an optional overnight incubation step

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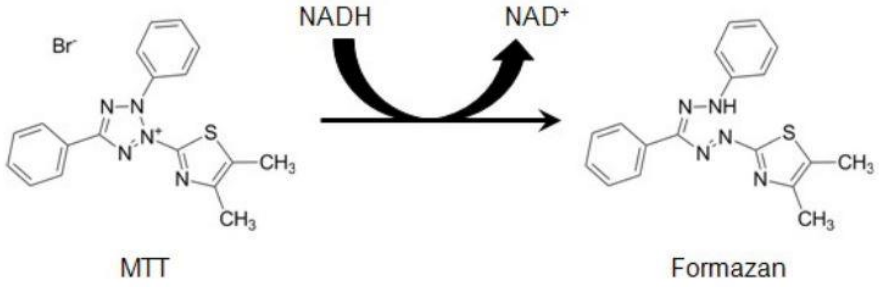
INTRODUCTION

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, Chondrex, Inc. introduces 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 (Cat # 6034) which employs a simple method to assay cell viability and cell density 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
Stock Solubilizing Solution (60342)	1 bottle	50 ml	Room Temperature
0.5M Acetic Acid (90625)	1 bottle	20 ml	Room Temperature

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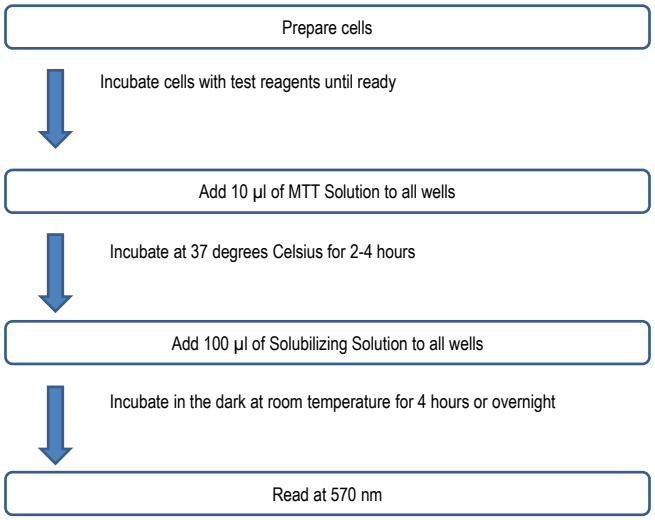
NOTES BEFORE USING ASSAY

NOTE: It is recommended that the standard and samples be run in duplicate.

ASSAY OUTLINE



ASSAY PROCEDURE

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 an appropriate amount, 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.

5. Prepare the solubilizing solution by mixing the Stock Solubilizing Solution and the 0.5M Acetic Acid at a 10: 1 ratio. For example, mix 1 ml of the Stock Solubilizing Solution and 100 µl of the 0.5M Acetic Acid to make the final solubilizing solution.

6. Add 100 µl of the final solubilizing solution to each well.

7. Mix the media and the solubilizing solution in the wells by pipetting or tapping the plate.

8. Incubate the plate in a dark place at room temperature for 4 hours or overnight.

9. Read absorbance at 570 nm.

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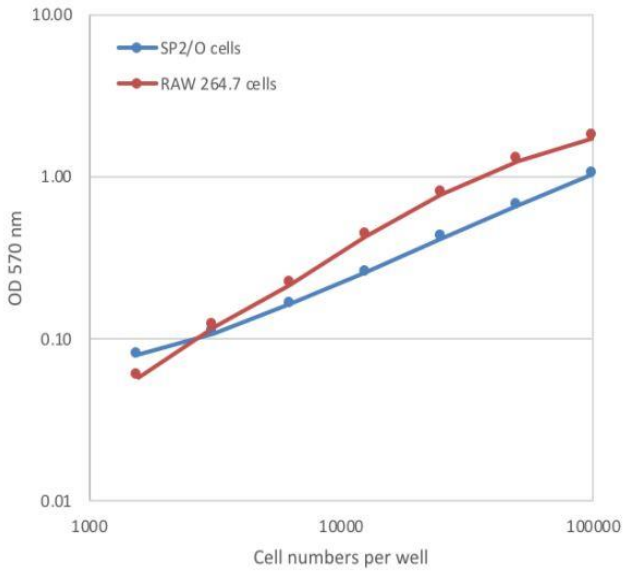
CALCULATING RESULTS

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 4 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.

TROUBLESHOOTING

For frequently asked questions about assays and ELISAs, please see Chondrex, Inc.'s Assay FAQ for more information.

REFERENCES

1. P. 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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