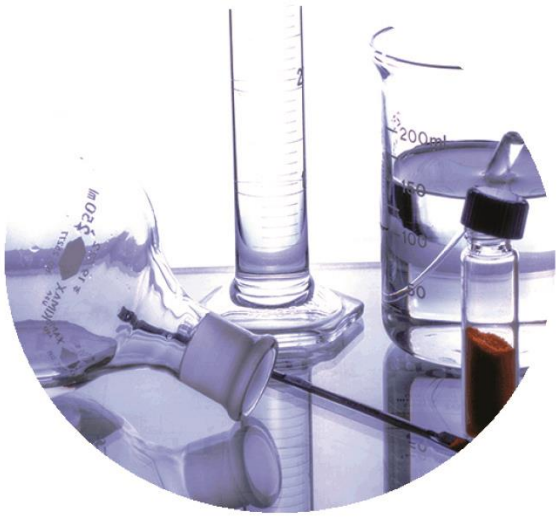

Assay kits

MTT – Cell Proliferation Kit

MTT- Cell Prolliferation kit

Protocol

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IMPORTANT NOTES – Before you begin

- ✓ The MTT – Cell Proliferation Kit is a colorimetric assay for measuring the mitochondrial reductase activity in living cells that reduce MTT to formazan dyes giving a blue/purple color. It is based on the cleavage of membrane-permeable yellow tetrazolium salt MTT to formazan crystals by metabolically active cells (figure 1).

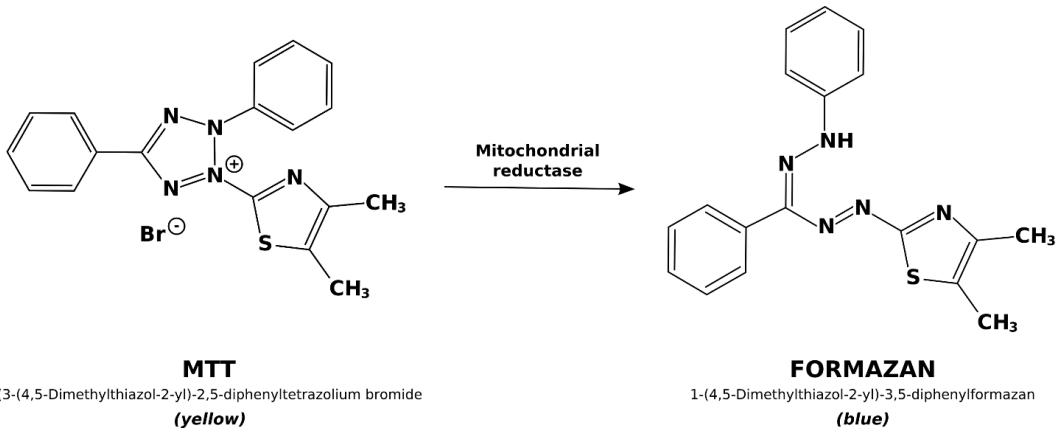

Figure 1: MTT metabolization to formazan salt in metabolically active cells

- ✓ A solubilization solution is then added to dissolve formazan into a colored solution. Spectrophotometric measurement of MTT-formazan at 570 nm allows quantitation of cell viability. Reagents used yield low background absorbance; a strong correlation between cell number and signal produced exists, allowing an accurate measurement of cell viability.

- ✓ **This kit is designed for spectrophotometric quantification of cell growth, viability and proliferation and can be used as a direct indicator of cytotoxicity and apoptosis.**

- ✓ This MTT – Cell Proliferation Kit is: Fast and Easy, Ready-to-use, Accurate, Economical and Stable under storage conditions.

For additional information and protocols (optimization, scaling, co-transfection…) tips, troubleshooting or other applications

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Any questions?

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Package content	MT01000 MTT reagent 10X :10 x 1 mL Solubilization Solution (ready to use): 1 x 100 mL Number of assays (96-well plate): 1000
Shipping conditions	The kit is shipped at room temperature.
Storage conditions	Upon receipt and for long-term use, store all reagent tubes at the indicated storage conditions MTT reagent 10X :-20°C Solubilization Solution (ready to use): +4°C
Shelf life	1 year from the date of purchase when properly stored and handled
Important notice	For research use only. Not for use in diagnostic procedures.

1. Usage

1. Plate the cells from 250 to 100 000 per well* and follow your experimental protocol.
2. Perform the MTT assay to determine cell viability.
3. Incubate 30 minutes to 4 hours until purple crystals are formed
4. Solubilize precipitates and measure absorbance at 570 nm.
5. Determine viability.

* number of cells depend on cell type (adherent/suspension), proliferation rate and metabolic activity.

2. Reagent preparation

- Dilute the 10X stock solution of MTT with sterile 1X PBS (pH 7.4). It is recommended to prepare fresh working solution each time the assay is performed.
- Excess of 1X solution can be returned at 4°C for short term storage (week) or -20°C for medium term storage (< 3 months).

Resulting solution should have a bright yellow color. Avoid repeated freeze/thaw cycles.

3. General Protocol for adherent cells in 96-well plate format

NOTE: 96 multiwell plates are the optimal vessel type for MTT method. Nevertheless it can be adapted to any vessel format, please refer to the table below.

1. Seed cells in a 96-well plate under standard culture conditions.
2. Carry out experiment by adding chemical compounds or biological agents to cells.

NOTE: at least two wells of cells should be kept untreated for viability control.

3. Wash cultured cells with 37°C pre-heated PBS.
4. Prepare **MTT working solution** as described in section 2.2.
5. Remove PBS and add 100 µL of **MTT working solution** to each well.
6. Incubate from 30 min to 4 h at 37°C (time depends on cell type/density/activity).
7. At the end of incubation time, add 100 µL of **solubilization solution**. Pipette up and down several times to make sure the converted dye is completely dissolved.
8. Measure the absorbance of the converted dye on a plate reader at 570 nm and a reference wavelength at 650 nm.
9. Calculate the signal sample: OD_{570}-OD_{650} and express viability as a percentage of control cells.

4. General Protocol for suspension cells

1. Seed cells in a 96-well plate under standard culture conditions.
2. Carry out experiment by adding chemical compounds or biological agents to cells.

NOTE: at least two wells of cells should be kept untreated for viability control.

3. Prepare **MTT working solution** as described in section 2.2.
4. Directly add 100 µL of **MTT working solution** to each well.
5. Incubate from 30 min to 4 h at 37°C (time depends on cell type/density/activity).
6. At the end of incubation time, add 100 µL of **solubilization solution**. Pipette up and down several times to make sure the converted dye is completely dissolved.
7. Measure the absorbance of the converted dye on a plate reader at 570 nm and a reference wavelength at 650 nm.
8. Calculate the signal sample: OD_{570}-OD_{650} and express viability as a percentage of control cells.

5. General Protocol for larger samples

NOTE: This protocol is suitable to test viability on aliquots of trypsin-suspended adherent cells from large experiments or on suspension cells in large volumes.

1. Culture adherent or suspension cells as required.
2. Carry out experiment by adding chemical compounds or biological agents to cells.
3. For adherent cells: after trypsinization and washing, re suspend adherent cells. Take an aliquot of re-suspended adherent cells or suspension cells.
4. Transfer 250 µL of each sample in a 1.5 mL tube
5. Prepare **MTT working solution** as described in section 2.2.
6. Directly add 250 µL of **MTT working solution** to each tube.
7. Incubate from 30 min to 4 h at 37°C (time depends on cell type/density/activity).
8. At the end of incubation time, add 250 µL of **solubilization solution**. Pipette up and down several times to make sure the converted dye is completely dissolved.
9. Centrifuge at 13.000 rpm for 2 min and transfer 100 µL of supernatant in two wells of a 96-well plate.
10. Measure the absorbance of the converted dye on a plate reader at 570 nm and a reference wavelength at 650 nm.
11. Calculate the signal sample: OD_{570}-OD_{650} and express viability as a percentage of control cells.

Volumes of solution recommended for various culture dishes are listed in the subsequent table.

Type of culture dish	1X MTT working solution	Solubilization Buffer
96-well plate	100	100
24-well plate	250	250
12-well plate	500	500
6-well plate	1000	1000

NOTES

NOTES

Additional products

- **OZBlue Cell Viability kit** for cell viability measurement
- **ROS Detection Assay Kit** for measuring Reactive Oxygen Species activity within your cells

Purchaser Notification

Limited License
The purchase of the MTT-Cell Proliferation Kit grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in this protocol). This reagent is intended for in-house research only by the buyer. Such use is limited to the transfection of nucleic acids as described in the product manual. In addition, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences. Separate licenses are available from OZ Biosciences for the express purpose of non-research use or applications of the MTT-Cell Proliferation Kit. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact us at OZ Biosciences. Buyers may end this License at any time by returning all MTT-Cell Proliferation Kit reagents and documentation to OZ Biosciences, or by destroying all D-Luciferin components. Purchasers are advised to contact OZ Biosciences with the notification that a MTT-Cell Proliferation Kit is being returned in order to be reimbursed and/or to definitely terminate a license for internal research use only granted through the purchase of the kit(s). This document covers entirely the terms of the MTT-Cell Proliferation Kit research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.

Product Use Limitations
MTT-Cell Proliferation Kit and all of its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the use of the kit components by following proper research laboratory practices.

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Rev. 06/2024