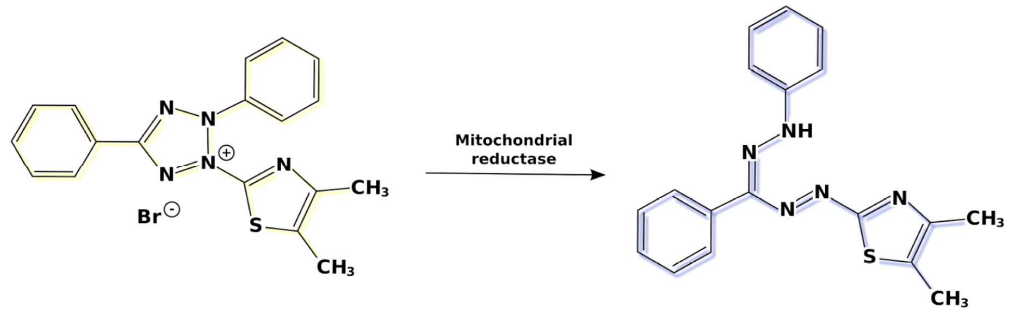
MTT Cell Proliferation Kit

INSTRUCTION MANUAL



MTT
(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
(yellow)

FORMAZAN
1-(4,5-Dimethylthiazol-2-yl)-3,5-diphenylformazan
(blue)

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MTT – Cell Proliferation Kit

Instruction Manual

Ready-to-use assay system for measurement of viability and proliferation rates based on spectrophotometric quantification of mitochondrial activity in living cells.

Catalog Number: MT01000

For all other additional information, do not hesitate to contact our dedicated technical support (tech@ozbiosciences.com).

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1. Technology

1.1. Description

Congratulations on your purchase of the MTT – Cell Proliferation Kit!

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).

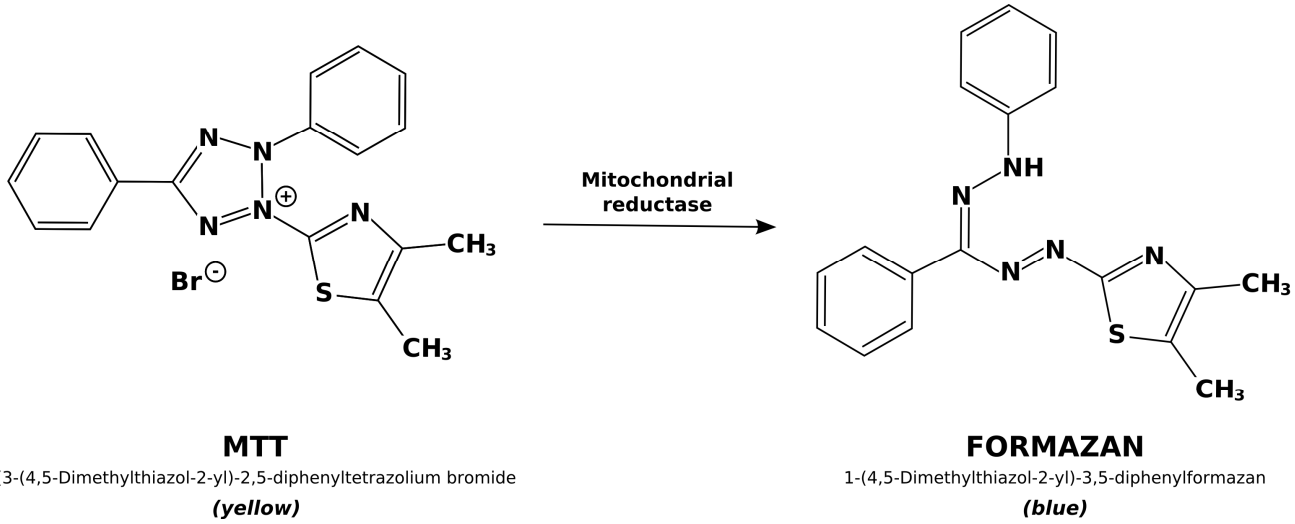

Figure 1: MTT metabolization to formazan salt in metabolically active cells

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.

1.2. Kit Contents

The kit contains sufficient reagents to perform 1000 assays in 96-well plate format.

Components	Quantity	Storage
MTT reagent 10X	10 x 1 mL	-20°C
Solubilization Solution (ready to use)	1 x 100 mL	4°C

Stability and Storage

Storage	Upon receipt and for long-term use, store all reagent tubes at the indicated storage conditions (see table above). Kit's components are stable for at least 1 year at the recommended storage temperature.
Shipping condition	Room Temperature

2. Applications and Protocols

2.1. 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.

2.2. 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.

2.3. 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.

2.4. 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.

2.5. 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

3. Related Products

Description
MAGNETOFECTION TECHNOLOGY
Super Magnetic Plate *(standard size for all cell culture support)* Mega Magnetic plate *(mega size to hold 4 culture dishes at one time)*
Transfection reagents:
PolyMag Neo (*for all nucleic acids*)
Magnetofectamine™ (*for all nucleic acids*)
NeuroMag *(dedicated for neurons)*
SilenceMag *(for siRNA application)*
Transfection enhancer:
CombiMag *(to improve any transfection reagent efficiency)*
Viral Transduction enhancers:
ViroMag (*to optimize viral transduction*)
ViroMag R/L *(specific for Retrovirus and Lentivirus)*
AdenoMag *(for Adenoviruses)*
LIPOFECTION TECHNOLOGY (LIPID-BASED)
Lullaby (*siRNA transfection reagent)*
DreamFect Gold *(Transfection reagent for all types of nucleic acids)*
VeroFect *(for Vero cells)*
FlyFectin *(for Insect cells)*
i-MICST TECHNOLOGY
Viro-MICST (*to transduce directly on magnetic cell purification columns)*
3D TRANSFECTION TECHNOLOGY
3Dfect *(for scaffolds culture)* / 3DfectIN *(for hydrogels culture)*
RECOMBINANT PROTEIN PRODUCTION
HYPE-5 Transfection Kit (*for **H**igh **Y**ield **P**rotein **E**xpression)*
PROTEIN DELIVERY SYSTEMS
Ab-DeliverIN *(delivery reagent for antibodies)* Pro-DeliverIN *(delivery reagent for protein in vivo and in vitro)*
PLASMIDS PVECTOZ
pVectOZ-LacZ / pVectOZ-SEAP / pVectOZ-GFP / pVectOZ-Luciferase
ASSAY KITS
Bradford – Protein Assay Kit MTT cell proliferation kit β-Galactosidase assay kits (CPRG/ONPG)
BIOCHEMICALS
D-Luciferin, K^+ and Na^+ 1g X-Gal powder 1g / G-418, Sulfate 1g

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Purchaser Notification

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