

1. Identification

Product Name: MTT Assay Kit

Catalog no: CCDK002
(1000 reactions and 2500 reactions)

2. Description

- ✓ 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).

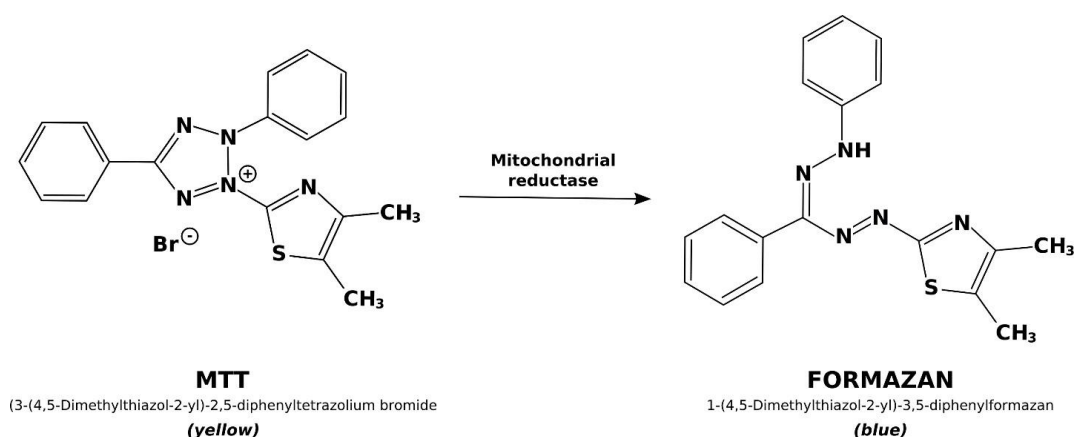


Figure 1: MTT metabolization to formazan salt in metabolically active cells

- ✓ 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.

3. Kit Components

Sl.no	Description	Content		Storage
		1000 reactions	2500 reactions	
1	MTT reagent, 1x Conc. Ready-to-use solution, non-sterile. • MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-phenyltetrazolium bromide) labeling reagent. • 5 mg/ml in Assay buffer.	5ml x 2 no's	5ml x 5 no's	Store at -15 to -25°C. • <i>Keep protected from light.</i> • <i>Repeated freeze-thaw cycles do not affect product stability. If precipitates form during shipment or storage, warm the container to +37°C and mix thoroughly.</i> After thawing, store the MTT reagent protected from light at +2 to +8°C for up to 4 weeks. Aseptically filter using 0.22µm filter and aliquot 1ml/vial and use it as required.
2	Solubilization buffer, 1x Conc.	60 ml x 2 no's	60 ml x 5 no's	Store at 2 to 8°C. • <i>Precipitates will be observed during shipment or storage, thaw the container in a 37°C water bath and mix thoroughly until the solution becomes clear. Repeated freeze-thaw cycles do not affect product stability.</i> • <i>Solution contains a detergent avoid frothing.</i>

4. Applications

The Cell Proliferation Kit I (MTT) can be used in a variety of applications:

- Designed for the spectrophotometric quantification of cell growth and viability without the use of radioactive isotopes.
- Measurement of cell proliferation in response to growth factors, cytokines, and nutrients.
- Useful for the measurement of cytotoxicity. Examples include the quantification of tumor necrosis factor-α or -β effects or macrophage-induced cell death and the assessment of cytotoxic or growth inhibiting agents, such as inhibitory antibodies.
- For the replacement of the radioactive [⁵¹Cr]-release cytotoxicity assay, protocols using MTT have been developed. The MTT assay is as sensitive as the radioactive method, but shows a significantly lower background, especially after long-term incubation.

DATA SHEET

Rev01

- The MTT assay can also be used to study cell activation.

ASSAY PROTOCOL

An overview of the steps is shown in the following table:

Step	Description	Volume/well [μl]	Time [Hours]	Temperature [°C]
1	Perform tissue culture using 96-well, tissue-culture grade, flat-bottom microplates.	100	24 – 96	+37
2	Add MTT reagent and incubate in a humidified atmosphere.	10	4	+37
3	Add Solubilization buffer and incubate in a humidified atmosphere.	100	4 - 18	+37
4	Evaluate microplate using an ELISA reader at 550 to 600 nm with a reference wavelength of >650 nm.			

Note: If for the initial incubation of the cells, a larger volume of culture medium is required, increase the amount of MTT reagent correspondingly, for example, 20 μl MTT reagent when cells are cultured in 200 μl culture medium

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

- Grow cells in microplates (tissue-culture grade, 96 wells, flat bottom) in a final volume of 100 μl culture medium per well, according to the media needs of the cells, in a humidified atmosphere, such as +37°C, 5 to 6.5% CO₂.
– The incubation period of the cell cultures depends on the particular experimental approach and on the cell line used for the assay. For most experimental setups, incubate cells for 24 to 96 hours.
- For adherent cells, remove the medium and replace it with 100 μL of fresh culture medium. For non-adherent cells, centrifuge the microplate, pellet the cells, carefully remove as much medium as possible and replace it with 100 μL of fresh medium.
- After the incubation period, add 10 μl of the MTT labeling reagent (final concentration 0.5 mg/ml) to each well.
- Incubate the microplate for 4 hours in a humidified atmosphere, such as +37°C, 5 to 6.5% CO₂.
- Add 100 μl of the Solubilization buffer into each well.
- Allow the plate to stand 4-18 hours in the incubator in a humidified atmosphere, such as +37°C, 5 to 6.5% CO₂.
- Check for total solubilization of the purple formazan crystals and measure the spectrophotometrically absorbance of the samples using a microplate (ELISA) reader. The wavelength to measure absorbance of the formazan product is between 550 and 600 nm according to the filters available for the ELISA reader used. Use a reference wavelength >650 nm.

2. Quick Protocol Option

To shorten the time of the assay it is possible to use DMSO (not provided) as a solubilizing agent to dissolve the formazan.

- After labeling the cells with MTT, as described above, remove all but 25 μL of medium from the wells. For non-adherent cells it may be necessary to first centrifuge the plates to sediment the cells.
- Add 50 μL of DMSO to each well and mix thoroughly with the pipette.
- Incubate at 37°C for 10 minutes.
- Mix each sample again and read absorbance at 550 nm not 600 nm, as above.

5. PRODUCT USE LIMITATION

Product Use Limitations This product is developed, designed, and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Safety Information When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.