MTT-Cell Based Proliferation/Toxicity Assay

Cat No. MTT-1200

Precautions: MTT is carcinogenic. Avoid direct contact. Use gloves and eye protection.
For research use only. Not for human or diagnostic use.

Storage: -20°C, dark

Overview: MTT Cell-based Toxicity Assay System Kit
The key substrate of the kit is: 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide or MTT. MTT is a yellowish solution when dissolved in balanced salt solutions without phenol red and is taken up by cells due to its net positive charge. The tetrazolium ring of MTT (yellow) is reduced to purple formazan crystals by intracellular NAD(P)H-oxidoreductases. The formazan crystals are insoluble in aqueous solution, but become solubilized in the provided Solubilization Buffer. After solubilization, the resulting purple solution is spectrophotometrically measured.

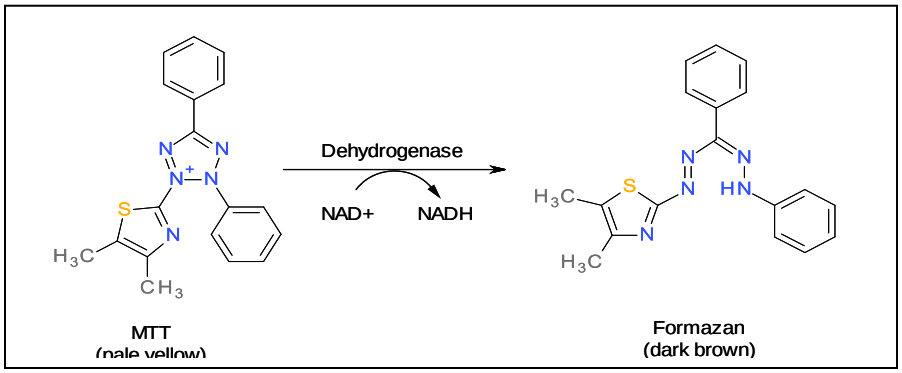

Figure 1: Chemical structure of MTT and its subsequent product. MTT is converted to Formazan.

Kit Contents

Catalog Number	Item	Quantity	Storage
MTT-R1	MTT Reagent (powder)	3 vials/20 mg per vial	-20°C protect from light
AB-MTT1	Assay Buffer (10 ml)	1 bottle	Ambient Temperature
SB-MTT1	Solubilization Buffer (125 ml)	1 bottle	Ambient Temperature

Procedure: 96 Well Format

MTT Toxicity Assay Preparation:
The MTT cytotoxicity method is appropriate for cell viability measurements in multiwell plate format, enabling high throughput screening. For best results, cells in log phase of growth should be employed and the final cell number should not exceed 10^6 cells/cm^2. Each experiment should include a blank containing all of the reagents in a well without cells.

Note: A typical experimental plate will include wells without cells, wells with cells treated with experimental reagents and wells with untreated cells. It is recommended that each treatment should be conducted in triplicate.

1. Seed cells in a 96-well plate at a density of $5x10^3$-$5x10^4$ cells/well in 100 μl of culture medium until cells reach 70-80% confluency. Culture the cells in an incubator for 24-72 hours.
2. After cell culture, transfer the plate of cells into a laminar flow hood or other sterile work area.
3. Reconstitute each provided bottle of MTT (MTT-R1) to be used with 4 ml of Assay Buffer (AB-MTT1) that is included with the kit. (one 96 well dish requires 960 μl of assay solution).
4. Once reconstituted, MTT solution can be stored at 4°C for 1 month or -20°C for 6 months.
5. For frozen MTT solution, thaw at 37°C for 5-10 minutes before use. (prevent repetitive freeze-thaw cycle)
6. Add 10 μl of MTT Reagent (prepared above) directly to the medium of each well in a 96 well dish.
7. Designate only one well for blank and add 100 μl medium and 10 μl MTT solution in this well.
8. Mix gently for one minute on an orbital shaker or gently tap the plate for a few seconds.
9. Incubate the cells for two hours in the incubator. Dark brown formazan crystals are formed in the cells. For higher cell density (> 10^5 cells per well), the incubation time can be shortened accordingly.
10. Warm up the Solubilization Buffer (SB-MTT1) in a 37°C water bath for 10 min before use.
11. Remove the culture medium from each well (including blank) carefully not to disturb the cell monolayer.
12. Add 100 μl of provided Solubilization Buffer (SB-MTT1) to each well (including blank) with mixing but avoid introducing bubbles in the solution. This solution will dissolve the formazan crystals and produce yellowish solution.
13. Foil wrap and rotate the plate at room temperature on an orbital shaker for 15 min. (If cell density is too high, solubilization time should be increased accordingly). Adjust the speed of the orbital shaker to assure crystal solubilization but prevent spillover into neighboring wells.
14. Measure the absorbance to each sample at 570 nm using a microplate reader.
15. The absorbance of the control group and the treatment group should be compared for quantitation of toxicity.