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# MTT Assay

Forked from MTT Assay

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Alexandria White[1]

[1]Emory University

Alexandria White
Emory University





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**Protocol status:** Working
**We use this protocol and it's working**

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## Abstract

The CyQUANT MTT Cell Viability Assay utilizes the well-established and widely used MTT reagent to determine mammalian cell viability. The redox potential in active mammalian cells reduces MTT to a strongly pigmented formazan product. After solubilization, the absorbance of the formazan can be measured with a microplate absorbance reader. The CyQUANT MTT Cell Viability Assay is a complete, optimized kit that provides all the reagents necessary for the detection of mammalian cell viability. The kit provides a sufficient amount of material for ~1000 assays.

Measuring changes to cell viability is a fundamental method for assessing cell health, determining genotoxicity, and evaluating anti-cancer drugs. Several methods can be used for such determinations, but procedures using colorimetric indicators provide a rapid and cost-effective method for determining changes to mammalian cell viability. Among the various colorimetric viability assays, the MTT assay is a well-established and popular assay.

The redox potential in viable mammalian cells causes the water soluble MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to convert to an insoluble formazan product. After solubilization of the formazan with the included SDS (sodium dodecyl sulfate) reagent, the concentration of the colorimetric probe is determined by an optical density measurement at 570 nm.

The CyQUANT MTT Cell Viability Assay provides a simple method for determination of mammalian cell viability using standard microplate absorbance readers. Simply prepare the MTT reagent, add it to the cells, solubilize the resulting formazan, and determine the optical density using a standard microplate reader. The CyQUANT MTT Cell Viability Assay provides the necessary materials to perform 1,000 individual tests using standard 96-well microplates.

1 Prepare MTT stock solution (12 mM)
1. Add 1 ml of sterile PBS to 5 mg of MTT (10x).
2. Vortex until dissolved. The undissolved particulate material may be removed by filtration or centrifugation.
3. Solution will be sufficient for 100, 10 µl tests, and be viable for 4 weeks, stored at 4 °C

2 Seed wells with 5,000 - 10,000 cells per well (96 total wells) and grow for 24-96 hours.

3 Check for positive growth via microscope observation before performing experimental treatment of cells.

4 The killing agent is typically added 24 hours after original seeding.

5 After waiting for the treatment time, remove media and replace with 100 µl of 37ºC media in each well.

Media may be DMEM with 10% FBS. Preferably without phenol red.

6 Add 10 µl of MTT stock to each well.

7 Incubate at 37 °C for 3-4 hours.
- High cell densities >100,000, incubation time may be shortened to 2 hours.

8 Remove 85 µl of medium from each well (25 µl of medium should remain).

9 Add 50 µl of DMSO to each well and mix thoroughly via pipette.

10 Incubate at 37 °C for 10 minutes.

11 Read absorbance at 540 nm.