

# Cell Growth Determination Kit

Cat No. MTT-M-001-500

Size: 500 Tests

MTT Solution stored at -20°C

Formazan Dissolving Solution stored at room temperature

**MOLEQULE-ON**<sup>®</sup>

Components	Quantity
MTT Solution	5ml
Formazan Dissolving Solution	60ml

## Description

The kit is designed for the spectrophotometric measurement of cell growth as a function of mitochondrial activity in living cells. MTT could measure the activity of living cells via mitochondrial dehydrogenase activity with simple, accurate and reproducible functions. Dehydrogenases in mitochondria can deoxidize MTT to crystalline purple product Formazan. The crystals can be dissolved in specific solvent. Use Microplate Absorbance Reader to measure absorbance near 490nm wavelength. An increase in cell number results in an increase in the amount of MTT Formazan formed and an increase in absorbance.

For best results, cell numbers should be determined during log growth stage. Each test should include a blank containing complete culture medium without cells.

## Protocol

1. Harvest cells (log growth stage) by centrifugation. Add 180µl of cell suspension into each well of 96-well plate. Adjust the concentration of cell suspension to 3-10 x 10<sup>3</sup> cell/well.
2. Return cultures to incubator (37°C, 5% CO<sub>2</sub>) and incubate for 6 to 24 hours.
3. Add appropriate concentrations of toxicant into the culture media in the plate. Incubate the plate for an appropriate length of time in the incubator.
4. Remove supernatant, add 90µl fresh culture medium then add 10µl MTT Solution then continue to culture for 4 hours.
5. Remove supernatant, add 110µL Formazan Dissolving Solution in each cell, slowly stirring in a gyratory shaker for 10 minutes, make the crystals fully dissolved.
6. Set the zero adjustment wells (Culture medium, MTT Solution, Formazan Dissolving Solution), control wells (Cell, solvent of the same concentration toxicant, Culture medium, MTT Solution, Formazan Dissolving Solution), 3 replications of each group.
7. Measure the absorbance at 490 nm using a microplate reader.

## Notes

- Uneven evaporation of culture fluid in 96 well plates may cause erroneous results. The outermost circle is most easy to evaporate, so you can discard them.
- MTT Solution is stable when stored frozen. Storage at 2-8°C may result in decomposition and yield erroneous results. MTT Solution should be store avoiding light, as MTT will invalidate when expose to light for a long time.
- Development of dark color or formation of crystals indicate product deterioration. If the Formazan Dissolving Solution is frozen or precipitate, dissolve in 37 °C water bath, the precipitate must be dissolved and mixed before use.