MTT Assay of Cell Numbers after Drug/Toxin Treatment

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[Abstract] MTT assay is a colorimetric method for measuring the activity of enzymes in living cells that reduce MTT to formazan dyes, giving a purple color. It is commonly used to determine cytotoxicity of potential medicinal agents and toxic materials, since these types of materials are expected to stimulate or inhibit cell viability and growth. Here, a general protocol is described to carry out an MTT assay on different types of cells.

Materials and Reagents

1. Raw264.7, MCF-7 or Hela cells
2. Thiazolyl blue tetrazolium bromide (MTT) (Sigma-Aldrich, catalog number: M5655)
3. DMSO
4. DPBS (Life Technologies, Invitrogen™, catalog number: 14190-250)
5. General chemicals (Sigma-Aldrich)

Equipment

1. 96 well plate
2. Shaking table
3. Paper towels
4. Incubator

Procedure

1. Plate 500-10,000 cells in 200 µl media per well in a 96 well plate. Leave 8 wells empty for blank controls.
2. Incubate (37 °C, 5% CO_2) overnight to allow the cells to attach to the wells.
3. Add 2 µl of drug of interest dissolved in DMSO to each well. Place on a shaking table, 150 rpm for 5 min, to thoroughly mix the samples into the media.
4. Incubate (37 °C, 5% CO_2) for 1-5 days to allow the drug/toxin to take effect.

5. Make 2 ml or more of MTT solution per 96 well plate at 5 mg/ml in DPBS. Do not make a stock as MTT in solution is not stable long-term.
6. Add 20 µl MTT solution to each well. Place on a shaking table, 150 rpm for 5 min, to thoroughly mix the MTT into the media.
7. Incubate (37 °C, 5% CO_2) for 1-5 h to allow the MTT to be metabolized.
8. Dump off the media (dry plate on paper towels to remove residue if necessary).
9. Resuspend formazan (MTT metabolic product) in 200 µl DMSO. Place on a shaking table, 150 rpm for 5 min, to thoroughly mix the formazan into the solvent.
10. Read optical density at 560 nm and subtract background at 670 nm. Optical density should be directly correlated with cell quantity.

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References

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