

MTT assay for cell proliferation

MTT stock solution: 5mg/ml MTT (Promega) in RPMI-1640 without phenol red.
This solution is filtered through a 0.2 μ m filter and stored at 2-8°C.

MTT working solution:

1:10 dilution of the 5mg/ml stock (MTT in RPMI without phenol red).

1. Wash cultured cells with warm RPMI-1640 without phenol red.
2. Prepare MTT working solution.
3. Add MTT working solution into wells being assayed, for example 1.0ml for each well of 12-well plate. Incubate at 37°C for 30min to 3 hrs (this time depends on cell density and cell type).
4. At the end of the incubation period, the medium can be moved if working with attached cells.
5. The converted dye is solubilized with 1ml acidic isopropanol (0.04 M HCl in absolute isopropanol). Pipette up and down several times to make sure the converted dye dissolves completely.
6. Transfer the dye solution with the cells into a 1.5 ml eppendorf tube and centrifuge at 13,000 rpm for 2 min.
7. Transfer the supernatant into a new eppendorf tube. Absorbance of the converted dye is measured at a wavelength of 570nm with background subtraction at 650nm. For the measurement, use Beckman DU-600 Spectrophotometer and disposable plastic cuvettes.