

MTT ASSAY

Principle of assay:

This is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with an organic solvent (eg. isopropanol) and the released, solubilised formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells.

Materials:

PBS

MTT (5 mg/ml in PBS) – filter and keep dark, prepare freshly

Acidic isopropanol (0.1N HCl in absolute isopropanol)

96-well plate (flat bottom)

Procedure:

1. Plate cells ($10^4 - 10^6$ cells) in 200 μ l PBS in 96-well (flat bottom).
2. Add 20 μ l of MTT solution, mix well.
3. Incubate for 4h in 37C in dark.
4. Remove aliquot for analysis; add 200 μ l acidic isopropanol and mix well.
5. Incubate additional 1h in 37C in dark.
6. Read plate in ELISA Reader – measure OD in 570nm (background wavelength is 630nm).

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