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MTT assay

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

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Abstract

The MTT assay is based on the conversion of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide into purple formazan crystals by LIVINGcells, which determines the mitochondrial activity of these cells. Viable cells contain NADPH-dependent oxidoreductase enzymes which reduce MTT to formazan.1The insoluble formazan crystals are dissolved using a solubilization solution (e.g. acidified isopropanol, DMSO) and the resultant coloured solution is quantified by measuring the absorbance at 500-600nm using a multi-plate spectrophotometer. The darker the solution, the greater the number of viable, metabolically active cells2.Since for most cell populations, the total mitochondrialactivity would be proportional to the number of viablecells, this assay is widely used in characterizingthe cytotoxiceffects of drugs or nanoparticleformulationson immortalized cell line or primary cell cultures.

Day 0

1 Passage cells which are at 80-90% confluence. Perform the usual trypsinization procedure.

2 Seed 15,000 cells well (100uL) in complete DMEM (using 1X DMEM) in a 96 well plate. Make sure to fill the peripheral wells with 100uL of water. Only use the inner 60 wells for test wells. Seed cells in one plate solely for the test concentrations (10 concentrations, sextuplicates). Seed another plate just to keep nanoparticle-only, DMEM-only and negative controls.

Day 1

3 Remove expired media.

4 Without any PBS wash, replace the wells with 100uL of nanoparticle-containing media of different concentrations. Use complete medium for this step (more serum-free or reduced serum media)

Day 2

2h 16m 25s

5 Remove ALL media

6 Replace with 100uL of complete DMEM + MTT (MTT final concentration = 0.5mg/mL in DMEM)

7 After adding DMEM +MTT, shake the plates for 00:00:30 at 70rpm on an orbital shaker. 30s

8 Incubate wells with MTT for 02:00:00 at 37 °C 2h

9 Remove all media + MTT from wells

10 Add 100uL DMSO into all the wells and shake for 00:00:10 at 70rpm on an orbital shaker. 10s

11 Incubate for 00:15:00 at 37 °C 15m

12 Place the plates in a microplane reader. Shake the plate using the in-built shaker feature for at least 00:00:45 at 425 cpm (fast) using double orbital shaking method. Following that, take absorbance measurements of wells @ 570nm using a microplane reader 45s