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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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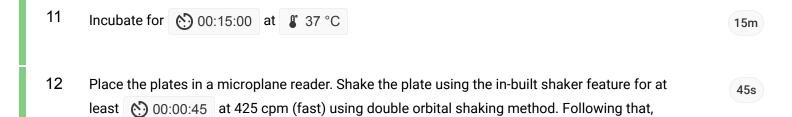
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Abstract

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)	
6 7	Replace with 100uL of complete DMEM + MTT (MTT final concentration = 0.5mg/mL in DMEM) After adding DMEM +MTT, shake the plates for 00:00:30 at 70rpm on an orbital shaker.	30s
_		30s 2h
7	After adding DMEM +MTT, shake the plates for 00:00:30 at 70rpm on an orbital shaker.	



take absorbance measurements of wells @ 570nm using a microplane reader