MTT Assay

Payam Amiri

Abstract

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Protocol

Step 1.

Prepare MTT stock solution (12 mM)

1. Add 1 ml of sterile PBS to 5 mg of MTT (5x).
2. Vortex until dissolved. The undissolved particulate material may be removed by filtration or centrifugation.
3. Solution will be sufficient for 100, 10 µl tests, and be viable for 4 weeks, stored at 4 °C

Step 2.

Seed wells with 5,000 - 10,000 cells per well (96 total wells) and grow for 24-96 hours.

Step 3.

Check for positive growth via microscope observation before performing experimental treatment of cells.

Step 4.

The killing agent is typically added 24 hours after original seeding.

Step 5.

After waiting for the treatment time, remove media and replace with 100 µl of 37ºC media in each well.

Media may be DMEM or 1640-RPMI with 10% FBS. Preferably without phenol red.

Step 6.

Add 25 µl of MTT stock to each well.

Step 7.

Incubate at 37 °C for 4 hours.

- High cell densities >100,000, incubation time may be shortened to 2 hours.

Step 8.

Remove 100 µl of medium from each well (25 µl of medium should remain).

Step 9.

Add 50 µl of DMSO to each well and mix thoroughly via pipette.

Step 10.

Incubate at 37 °C for 10 minutes.

Step 11.

Read absorbance at 570 nm.