Cyrusbioscience

MTT Assay Protocol

Reagent preparation and assay procedure for MTT assays to measure cell proliferation or cell cytoxicity.

Reagent preparation

Prepare MTT solution:

MTT is soluble in water (10 mg/mL), ethanol (20 mg/mL), and buffered salt solutions and culture media (5 mg/mL). We recommend using a 5 mg/mL solution in PBS. Mix by vortexing or sonication. Filter sterilize solution after adding MTT.

Store MTT solution at -20°C (stable for at least 6 months). Do not store at 20°C for more than a few days.

Prepare MTT solvent:

4 mM HCl, 0.1% NP40 in isopropanol

Assay protocol

1. Discard media from cell cultures. For adherent cells, carefully aspirate the media. For suspension cells, spin the 96 well plate at 1,000 x g, 4°C for 5 minutes in a microplate-compatible centrifuge and carefully aspirate the media.

   An alternative method is to add an equal volume of MTT solution to the existing media in the culture. Ensure that the same volume of existing media is present for each sample.
2. Add 50 µL of serum-free media and 50 µL of MTT solution into each well.
3. Incubate the plate at 37°C for 3 hours.
4. After incubation, add 150 µL of MTT solvent into each well.
5. Wrap plate in foil and shake on an orbital shaker for 15 minutes. Occasionally, pipetting of the liquid may be required to fully dissolve the MTT formazan.
6. Read absorbance at OD=590 nm. Read plate within 1 hour.

   Notes: Serum or phenol red present in the culture medium can generate background. If your sample contains serum or phenol red, set up sample background controls: 50 µL MTT reagent + 50 µL cell culture media (no cells).

   Prepare parallel well(s) as solvent control and use same volume of solvent as for the treated cells.

Data analysis

Cell proliferation assays

1. Average the duplicate reading for each sample.
2. Subtract the culture medium background from your assay reading. This is the corrected absorbance.
3. Amount of absorbance is proportional to cell number.

   For cell counting, a standard curve can be established with known cell number and fixed incubation times with the assay reagent.

Cell cytotoxicity assays

1. Average the duplicate reading for each sample.
2. Subtract the culture medium background from your assay readings. This is the corrected absorbance.
3. Calculate percentage cytotoxicity with the following equation, using corrected absorbance: % cytoxicity = (100 x (control - sample))