In Vitro Application

MTT Cell Growth Assay Protocol for 96-well and 384-well LifeGel Plates

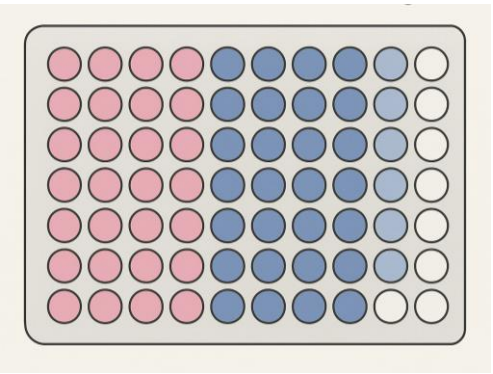
The MTT assay is a colorimetric method used to assess cell viability and metabolic activity. It relies on the ability of living cells to convert the yellow tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), into purple formazan crystals. The quantity of formazan generated is directly proportional to the number of metabolically active, viable cells. I also can be used to measure the cytotoxic effects of drugs or other substances on cells.

Key advantages:

- Simple and Relatively Fast: The MTT assay is a relatively simple and quick procedure.
- Sensitive: It can detect small changes in cell viability.
- Cost-Effective: It is a relatively inexpensive assay

MTT Cell Growth Assay protocol (Sigma-Aldrich).

MTT Cell Growth Assay is a colorimetric assay that can be used for either proliferation or complement-mediated cytotoxicity assays



Storage:
Maintain at 2-8°C for up to six months. The Reagent A / Solution B mixture is stable at 2-8°C for up to two weeks.

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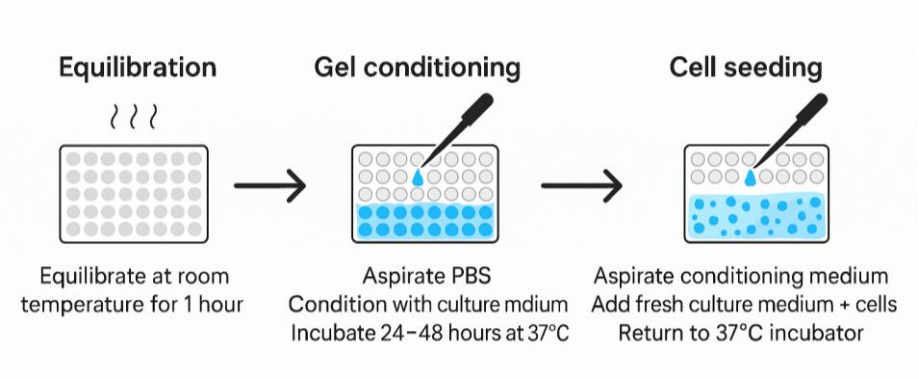
PROTOCOL:

Preparing 96- or 384-well LifeGel plates for MTT Cell Growth Assay

Materials required:

- 96- or 384-well LifeGel plate
- Pipette/multichannel pipettor
- Complete culture medium

1. **Equilibration**
   Remove LifeGel plates from the refrigerator 24 or 48 hours before seeding. Allow them to equilibrate at room temperature for approximately 1 hour.
2. **Gel conditioning**
   Aspirate the PBS from each well. Add 150 µl of culture medium for 96-well plates or 60 µl for 384-well plates to condition the gel. Incubate the plates at 37°C in a humidified incubator.
3. **Cell seeding**
   After 24 or 48 hours of conditioning, remove the plates from the incubator.
   Aspirate the conditioning medium from the wells.
   Add 150 µl (96-well) or 60 µl (384-well) of fresh culture medium containing cell suspension to each well.
   Include appropriate control wells as needed*.
   Return the seeded LifeGel plates to the 37°C incubator for cell culture.



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PROTOCOL:

MTT Cell Growth Assay for 96-well LifeGel Plates

Reagent Preparation

To prepare the working solution for 1,000 assays:

- Add 10 mL of Solution B to one vial of Reagent A.
- Mix thoroughly until fully dissolved.
- Sterile filter the solution and store it protected from light at 4°C.

Note: Dissolution may require overnight incubation. Do **not** apply heat. If undissolved crystals remain, adjust the pH by adding 1–2 drops of HCl. Under these conditions, the reagent (AB mixture) remains stable for several weeks.

Reagent Addition

- Remove assay plates from the 37°C incubator.
- Carefully aspirate 50 µL of medium from each well.
- Add 10 µL of the prepared AB (MTT) solution to each well of 96-well LifeGel plates.

Incubation

- Incubate plates at 37°C to allow MTT reduction by viable cells.
- Incubation time may vary depending on cell type and experimental conditions; however, 4 hours is generally sufficient.
- MTT is reduced to black, fuzzy formazan crystals that accumulate at the bottom of wells containing live cells.

Formazan Solubilization

- Add 10 µL of isopropanol containing 0.04 N HCl to each well.
- Mix thoroughly using a multichannel pipette to ensure complete dissolution of formazan.
- The acidic isopropanol shifts phenol red to yellow, minimizing background interference during absorbance measurement.
- A uniform blue solution indicates successful formazan solubilization.

Measurement

Within 1 hour of solubilization, measure absorbance using a plate reader:

- Test wavelength: 570 nm
- Reference wavelength: 630 nm

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Important Notes:
Precipitation of serum proteins may occur after prolonged exposure to acid/alcohol at room temperature. To minimize this:

- Chill plates to accelerate precipitation if needed.
- If a delay is necessary before measurement, store plates at 4°C *before* adding the acid/alcohol. Return to room temperature and add the solubilization reagent immediately before reading.

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