Protocol

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MTT Cell Proliferation Assay

Introduction

MTT is a yellow tetrazolium dye that turns purple when it is reduced to an insoluble formazan. This reduction is carried out by oxidoreductase enzymes that are dependent on NADH or NADPH inside cells. The level of active oxidoreductase enzymes is reflective of the cellular metabolic activity. After solubilizing the formazan produced from a cell, the absorbance can be measured and compared to the absorbance of formazan in a control solution to determine if cellular metabolic activity has increased or decreased. Thus, MTT can be used to assess cell proliferation or cytotoxicity of drugs.

The MTT assay is influenced by the growth phase of the cells and variation of metabolic activity amongst different cell types. Cell count should be taken during log phase. This protocol is for use with 96 well plates.

Materials

- MTT (Thiazolyl Blue Tetrazolium Bromide (MTT), GoldBio Catalog # T-030 [CAS 298-93-1, mw = 414.32 g/mol])
- PBS (PBS (Phosphate Buffered Saline) Tablets, GoldBio Catalog # P-271)
- Dimethylsulfoxide (DMSO) (Sterile Filtered DMSO, Ultra Pure, GoldBio Catalog # D-361 [CAS 67-68-5, mw. = 78.13 g/mol)

Method

Incubate cells

1. Add 100 µl of cells to each well and incubate for 2-3 days.

Note: Use a consistent cell density that is 5,000-10,000 cells per well.

Note: Incubation time will vary depending on cell line and cell density.

Prepare MTT stock solution

2. Dissolve 5 mg MTT in 1 ml 1X PBS. Sterilize by filtration.

Labeling cells with MTT

3. Add 10 µl of MTT stock solution to each well.

4. Incubate for 2-5 hours at 37°C.

Solubilizing the formazan

5. Carefully remove media from each well without disturbing cells.

6. Add 100 µl of DMSO to each well and mix by pipetting up and down.

Note: Be sure to add 100 µl DMSO to a well without cells as a blank.

7. Incubate at 37°C for 15 minutes.

Measure absorbance

8. Measure the absorbance at 570 nm immediately.

Tips

- MTT solution should be stored at 0°C and will be stable for 6 months. If solution changes color or forms crystals, dispose of it properly.

- Determine cytotoxicity of DMSO to the cells being tested. If DMSO is too toxic for accurate results, an alternative solution of 0.2% nonidet p-40 (NP-40) (or nonidet P-40 substitute) and 8mM HCl in isopropanol can be used to solubilize the formazan. If this solution is used, include it as a blank.

References

Carmichael, J., DeGraff, W. G., Gazdar, A. F., Minna, J. D., & Mitchell, J. B. (1987). Evaluation of a Tetrazolium-based Semiautomated Colorimetric Assay: Assessment of Radiosensitivity. *Cancer Research*, *47*(4), 943-946.

Liu, Y., Peterson, D. A., Kimura, H., & Schubert, D. (1997). Mechanism of cellular 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) reduction. *Journal of neurochemistry*, *69*(2), 581-593.

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