Protocol

TD-P Revision 2.0

Creation Date: 9/16/2015
Revision Date: 2/6/2019

XTT Cell Proliferation Assay

Introduction

XTT is a negatively charged, tetrazolium salt that turns orange when it is reduced to a soluble formazan dye. This extracellular reduction is carried out by electron transport across the plasma membrane of a living cell. Because the reduction occurs by electron transport from outside the cell, the use of an intermediate electron acceptor such as phenazine methosulfate is required for complete reduction of XTT. The amount of XTT reduced is reflective of the cellular metabolic activity. The absorbance can be measured and compared to the absorbance of a control solution of untreated cells to determine if cellular metabolic activity has increased or decreased. Thus, XTT can be used to assess cell proliferation or cytotoxicity of drugs. The XTT assay has been reported to be more sensitive than the MTT assay.

The XTT 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. The presence of superoxide may also affect results. This protocol is for use with 96 well plates.

Materials

- XTT (XTT Sodium Salt, GoldBio Catalog # X-200 [CAS 111072-31-2, mw = 673.52 g/mol])
- PBS (PBS (Phosphate Buffered Saline) Tablets, GoldBio Catalog # P-271)
- PMS (Phenazine methosulfate)

Method

Incubate cells

1. Add 100 µl of cells to each well and incubate for 2-3 days.
    a. Use a consistent cell density that is 5,000-100,000 cells per well.
    b. Incubation time will vary depending on cell line and cell density.
    c. Be sure to include a control of untreated cells and a blank of culture media without cells.

Prepare PMS and XTT solution

2. Prepare PMS solution by dissolving 3 mg PMS in 1 ml 1X PBS.

3. Prepare XTT solution by dissolving 4 mg XTT in 4 ml of cell culture medium.

Labeling cells with XTT

4. Add 10 µl of the PMS solution to the 4 ml of XTT solution to create the detection solution.

5. Immediately, add 50 µl of detection solution made in the previous step to each well.

6. Incubate for 2-5 hours at 37°C.

Measure absorbance

7. Place the reaction on a shaker for a short period of time to mix the dye in the solution.

8. Measure the absorbance at 450 nm immediately.

Tips

- XTT solution and PMS solution can each be stored at -20°C in the dark and should be stable for 9 months. If XTT solution changes color or forms crystals, dispose of it properly.
- A standard curve can be generated to determine optimal cell densities by performing the experiment with a range of known cell densities and measuring the absorbance at 450 nm. The optimal cell density will fall on the most linear part of the plot and have an absorbance below 1.0.
- If the absorbance of the blanks is high, the culture medium may contain a reducing agent and an alternative medium should be used.

References

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