CELL CULTURE and MTT INTERNSHIP (20 hrs.)

Cell culture is a laboratory technique that involves the growth of cells outside of their natural environment. In this technique, cells are grown in a controlled environment in a special growth medium, which provides nutrients and other factors necessary for their survival and growth. Cell culture is used in a wide range of applications same as MTT proliferation assay.

1. CELL CULTURE

Cell culture is necessary for the MTT assay because it allows for the isolation and manipulation of specific cell types, and the measurement of cell viability and proliferation in a controlled and reproducible environment.

A. Perform aseptic techniques, sterile handling of equipment, and cell passaging.

i. How to work with Autoclave, oven, alcohol, UV light, filters, laminar hood, centrifuge, pipettes.
ii. Media exchange and Cell passaging
iii. Cell counting via hemacytometer

2. MTT

The MTT assay, also known as the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, is a colorimetric method for measuring cell viability and proliferation. This assay is often used to evaluate the cytotoxic effects of drugs, chemicals, or other treatments on cells in culture.

A. Perform seeding, treating and measuring the effect of samples to the cells.

i. How to count and seed cells
ii. How to make a serial dilution of drugs
iii. How to setup a MTT test

3. SPECTROPHOTOMETERY

In the MTT assay, the MTT reagent is added to the cell culture medium and is converted to formazan by the dehydrogenase enzymes in viable cells. The formazan product is

insoluble and can be quantified spectrophotometrically by measuring the absorbance at a specific wavelength, typically 570-590 nm.

i. Perform spectrophotometer
ii. Know about blank, sample and control group

4. DATA ANALISIS

A. Calculation of the absorbance ratio of each well to the untreated control, which is used to calculate the percentage of viable cells.

B. Statistical analysis of the data to determine the significance of the differences between the treated and untreated cells.

C. Plotting the absorbance ratio values against the treatment concentrations to generate a dose-response curve, which can be used to determine the concentration of the treatment that results in a 50% reduction in cell viability (EC50).