General Aim

This experiment aims at visualizing the Bromodeoxyuridine (BrdU) incorporation as a thymidine analog into nuclear DNA in order to detect DNA synthesis in vitro using antibody probes and a fluorescent microscope.

Method

In Vitro Immunofluorescence Assay for detection of incorporated BrdU during DNA replication.

Learning Objectives (ILOs)

- Successfully handle the required instruments and consumables needed in the experiment.
- Check the confluence and count cells under the microscope.
- Dilute the cells to a specific count suitable for seeding in the 96-well plate.
- Calculate the concentration of tested chemicals and prepare the calculated doses in the cell culture medium.
- Aspirate the old medium and add the new medium containing the tested chemicals in the appropriate wells.
- Add the BrdU assay substrate solution to cells and read the results using the fluorescent microscope after incubation of cells with first anti-BrdU and secondary antibodies.

Theoretical Background/Context

- In genetics, genotoxicity describes the property of chemical agents that damages the genetic information within a cell causing mutations, which may lead to cancer. While genotoxicity is often confused with mutagenicity, all mutagens are genotoxic, whereas not all genotoxic substances are mutagenic.

Principle of Work

- 5-Bromo- 2’-deoxyuridine (Bromodeoxyuridine (BrdU)) is a thymidine analog that differs from thymidine in its substitution of bromine for a methyl group. BrdU competes with thymidine for incorporation into nuclear DNA during the S-phase of the cell cycle. Therefore, BrdU serves as a marker of DNA synthesis, and separate means such as counting mitotic figures may be employed to ensure accuracy with regard to cell division.

- After BrdU is incorporated into nuclear DNA, samples are fixed and incubated with anti-BrdU monoclonal antibodies and nucleases (or they are exposed to heat or other conditions which cause DNA denaturation). This denaturation is necessary to allow anti-BrdU monoclonal antibodies to gain access to the incorporated BrdU in single-stranded DNA. The sample is subsequently incubated with a fluorescent secondary antibody against the anti-BrdU antibody and visualized by a fluorescence microscope.