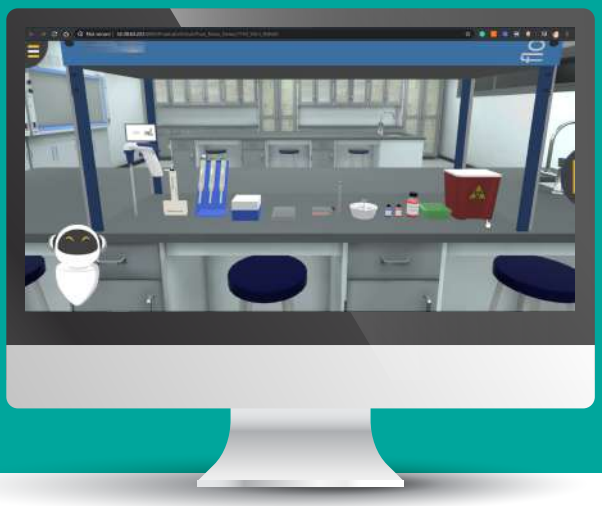
Biology | Toxicology

In Vitro Bromodeoxyuridin (BrdU) Assay



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

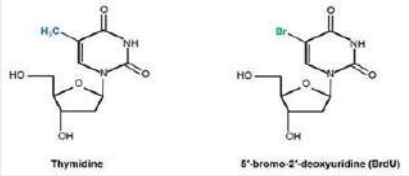
- In genetics, genotoxicity describes the property of chemical agents that damages the genetic information within a cell causing mutations, which may lead to cancer. While genotoxicity is often confused with mutagenicity, all mutagens are genotoxic, whereas not all genotoxic substances are mutagenic.

Theoretical Background/Context (Cont')

- The alteration can have direct or indirect effects on the DNA: the induction of mutations, mistimed event activation, and direct DNA damage leading to mutations. The permanent, heritable changes can affect either somatic cells of the organism or germ cells to be passed on to future generations. Cells prevent the expression of the genotoxic mutation by either DNA repair or apoptosis; however, the damage may not always be fixed leading to mutagenesis.

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.