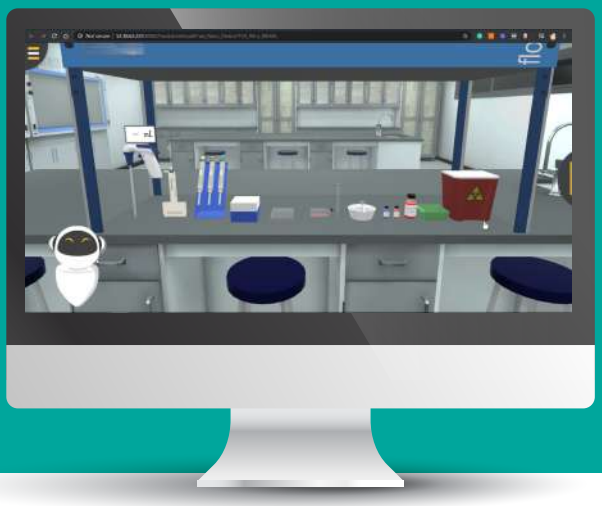
Biology | Toxicology

In Vitro 8-Hydroxydeoxy Guanosine (8-OHdG) Assay



General Aim

This experiment aims at visualizing the response of protein recruitment to DNA damage sites. DNA double-strand breaks (DSBs) induce the hydroxylation of 2'-deoxyGuanosine to form 8-Hydroxy, 2'-deoxy Guanosine (8-OHdG), and accumulate 8-OHdG which can then be detected as foci. The detection of 8-OHdG foci by immunostaining with antibodies that recognize 8-OHdG is an indicator of DSBs presence. This assay will describe the measurement of 8-OHdG immunostaining using a fluorescent microscope.

Method

In Vitro Immunofluorescence Assay for detection of 8-OHdG and DNA Damage

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 blocking solution, first and secondary antibodies to cells and read the results using the fluorescent microscope after incubation of cells.

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 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

- High levels of reactive oxygen species (ROS) might result in structural and/or genetic changes in cells modulating initial steps of carcinogenesis. As the accumulation of ROS and subsequent oxidative damage is commonly observed and widely studied in inflammatory diseases, thus, inflammation-induced ROS are linked to mutations in proto-oncogenes and tumor suppressor genes during cancer progression.
- 8-Hydroxy-2'-deoxy Guanosine (8-OHdG) is a single nucleotide base lesion and recognized as a biomarker of oxidative DNA damage. The detection of 8-OHdG is performed with a wide range of methodologies including immunofluorescence labeling which has certain benefits such as being performed with low numbers of cells in comparison to the analytical methods, while more cells is required to isolate relatively higher amounts of DNA for reliable quantitation of the damaged base.
- When one hundred cells are counted per sample, defined object criteria are applied to determine parameters such as number of cells analyzed, number of foci per cell, their sizes, volumes and the number of cells having or not having foci.