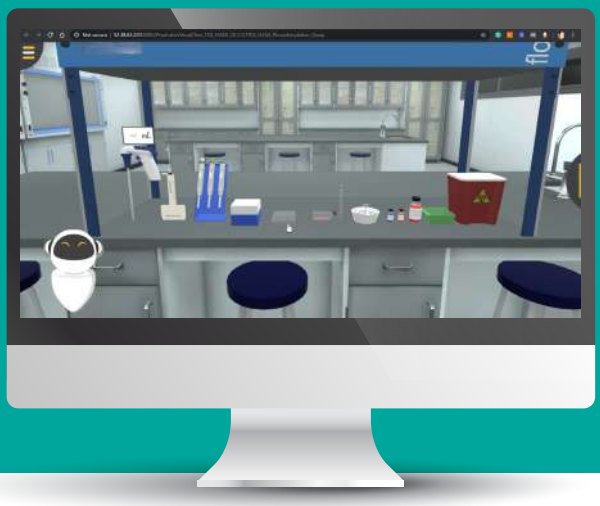
Biology | Toxicology

In Vitro Histone H2AX Phosphorylation Assay



General Aim

This experiment aims at visualizing the response of protein recruitment to DNA damage sites. DNA double-strand breaks (DSBs) and blocked replication forks induce the phosphorylation of H2AX at serine 139 (γH2AX), and accumulate γH2AX which can then be detected as foci. The detection of γH2AX foci by immunostaining with antibodies that recognize γ H2AX is an indicator of DSBs presence. This assay will describe the measurement of γH2AX immunostaining using a fluorescent microscope.

Method

In Vitro Immunofluorescence Assay for detection of Phosphorylated Histone H2AX 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 H2AX Phosphorylation assay substrate solution 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.
- 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

- DNA damage response is crucial to maintain the homeostasis of cells. Damage that remains unrepaired or incorrectly repaired may lead to genetic mutations, instability, and increased risk of carcinogenesis. One of the most serious sources of damage in cells, DNA double-strand breaks (DSBs) are often induced by a various source, including ionizing radiation and exposure to DNA- damaging chemical or environmental stress.

On the occurrence of DSB, cells initiate DNA response signaling and recruit DNA damage repair proteins to affected DNA sites to repair the altered DNA. After the formation of DSBs, H2AX, a variant form of histone H2A and is ubiquitously distributed throughout the genome, is rapidly phosphorylated on a serine 139 residue to create γH2AX. The γH2AX is phosphorylated in megabase regions of surrounding the DNA break site. Large numbers of γH2AX molecules can be visualized as foci in nuclear region by immunostaining with antibodies that recognize γH2AX. Monitoring of γH2AX foci formation by a fluorescent microscope is useful for detecting the incidence of DSBs.