Abstract

Cytotoxicity assays play a critical role in the identification of potential chemotherapeutic agents. Commonly used in vitro assays, such as the colorimetric MTT assay, measure the release of lactate dehydrogenase (LDH) and propidium iodide (PI) following treatment to generate an absorbance reading that is proportional to viable cells. These absorbance readings are often plotted on a log-scale to generate a dose-response curve, which is used to determine the concentration at which 50% of the cells are killed (IC50). However, these assays are limited in their ability to quantify cellular events in real-time and do not provide information on the cell viability or morphological changes occurring within the cell culture. Various methods have been developed to overcome these limitations, including automated image analysis tools that can monitor cell morphology, nuclear integrity, and permeability. However, these tools require expensive and specialized equipment, which limits their widespread adoption.

In this study, we evaluated the IncuCyte FLR System, a live-cell imaging platform that allows for real-time monitoring of cell viability, nuclear integrity, and permeability. The FLR System uses a combination of fluorescent dyes, including YOYO® and propidium iodide, to stain nucleic acids and nuclei, respectively. The FLR System also uses automated image analysis tools to quantify changes in cell morphology, nuclear integrity, and permeability. These changes are then plotted on a log-scale to generate a dose-response curve, which is used to determine the concentration at which 50% of the cells are killed (IC50).

Intra-assay Reproducibility

The IncuCyte FLR System was used to measure the cytotoxicity of various compounds on HEK 293 cells. The IC50 values were calculated using the Reed-Muench method, and the results were found to be highly reproducible. The FLR System also allows for the monitoring of cell viability, nuclear integrity, and permeability over time, which can be used to assess the effects of various compounds on cell viability and nuclear integrity.

Discrimination of Cytotoxic and Cytostatic Compounds

The FLR System was also used to discriminate between cytotoxic and cytostatic compounds. Cytotoxic compounds, which kill cells, were found to significantly reduce cell viability, nuclear integrity, and permeability. In contrast, cytostatic compounds, which arrest cells in the cell cycle, were found to reduce cell viability but not nuclear integrity or permeability.

Conclusions

The IncuCyte FLR System is a powerful tool for measuring cytotoxicity in vitro. It allows for real-time monitoring of cell viability, nuclear integrity, and permeability, which can be used to discriminate between cytotoxic and cytostatic compounds. The FLR System also allows for the monitoring of cell viability and nuclear integrity over time, which can be used to assess the effects of various compounds on cell viability and nuclear integrity. The FLR System is a valuable tool for research and development, and it is expected to become the standard method for measuring cytotoxicity in vitro.