アプリケーション
ノート:
LC01722000

Micronuclei Detection using a single channel HCS approach with Acridine Orange

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細胞遺伝毒性
小核誘発頻度
の自動測定
アクリジンオレンジ

- ArrayScanVTI
- Micronucleus BioApplication

Introduction

Micronucleus (MN) formation is a hallmark of genetic toxicity; as such, micronuclei are used as indicator of genotoxicity caused by drug candidates or enviromental toxins. Existing in vitro assays for micronuclei induction are time-consuming, require multiple trained personnel, can be subjective, and are not automated. Because of these constraints, it has been virtually impossible to test many compounds for this important indicator of genotoxicity. An automated in vitro assay that enables screning for indicators of genetic toxicity earlier in the development of therapeutic candidates would improve attrition rates and yield substantial financial gains for the pharmaceutical industry. We have developed an automated High Content Screening (HCS) solution with the Thermo Scientific Cellomics Micronucleus v3.0 BioApplication. It is a functional assay that measures the frequency of micronuclei (MN) to determine genotoxicity, as well as various indicators of cytotoxicity.

Materials

CHO-K1 cells were plated on 96 well collagen-I coated microtiter plates (BD Biosciences, San Jose, CA) and incubated for 18-22 hours. Cells were then treated with various doses of mitomycin C for 20 hours followed by treatment of 6 μg/mL cytochalasin B (Cal Biochem, San Diego, CA) for 28 hours. A fixation solution of methanol: glacial acetic acid was added to the media for five minutes and repeated after aspiration. Plates were washed three times with wash buffer and 20 μg/mL Acridine Orange (Invitrogen, Carlsbad, CA), a nucleic acid specific fluorescent dye specific for DNA and RNA, was added for three minutes. The plate washed twice and then placed on Thermo Scientific Cellomics ArrayScan VTI HCS reader. A single channel approach with a specific filter set optimiaed for Acridine Orange excitation and emmission was used for imaging. Follow up analysis using multiple channel configurations were conducted for comparison of results (data not shown).

Method of detection and analysis

The BioApplication identifies and selects only desired subpopulation based on analysis of images of fluorescently labeled cell generated by the ArrayScan VTI HCS reader. It then detects, distinguishes, and quantitates nuclei and micronuclei in the targeted subpopulation. (Figure 1). Unique to this BioApplication, the assay can be configured as a single channel rapid micronucleus detector, or as a two or three channel assay that permits robust flexibility when addressing complex cell lines and allows for more multiplexing reagents. High content information from the assay reports micronucleus frequency as the primary indicator of genotoxicity. Additionally, it can automatically determine a Proliferation Index as well as a ratio of multinucleated to mononucleated cells for monitoring cell-cycle delay, an early indicator of cytotoxicity. Addicionally, late stage cytotoxicity, through membrane permeability, can be assessed when used with the Cellomics Micronucleus HCS Reagent Kit.

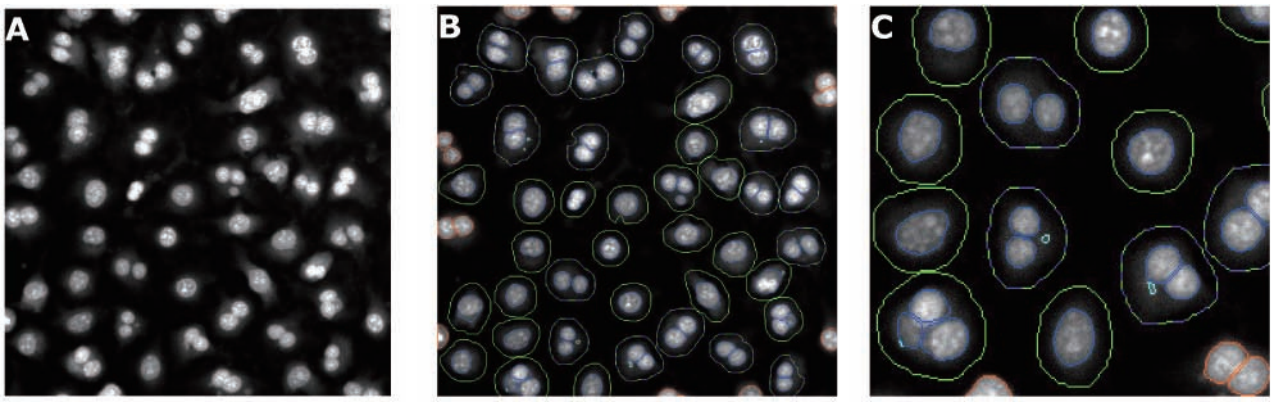

Figure 1. Imaging and detection of micronuclei.
Chinese Hamster Ovary Cells (CHO) stained with Acridine Orange and imaged with the ArrayScan VTI HCS Reader. (A) Gray-scale image of Acridine Orange labeled nuclei. (B) Algorithm overlay detecting included and excluded cells for analysis and micronuclei detection. (C) Enlarged section of panel B showing nuclear boundaries (royal blue overlay), selected cells (solid green overlay), Targeted cells (dashed purple and green overlay, and selected micronuclei cyan overlay).

Results

The ArrayScan VTI HCS Reader with the Micronucleus v3.0 BioApplication processed a minimum of 1000 Targeted (binucleate cells only) for analysis in each treatment. Micronuclei were detected in both a minimum and maximum treatment and a t-test was applied to determine if the populations were statiscally different from one another. Eight different doses of mitomycin C were then used to demonstrate a dose dependent effect and to determine an EC_{50} for mitomycin C. Results are presented in Figure 2.
The single channel micronucleus assay resulted in a t value of 14.79 with a $p \leqq 0.0001$ (Figure 2).
The EC_{50} was calculated at 260.0 ng/mL (Figure 3).
Additionally, the time to image and process this configuration is listed in Figure 4 total number of treatments per week is then extrapolated using an 18 hours screening day and was determined that more than 8600 wells could be processed week with this assay.

Conclusions

Micronuclei were detected using the ArrayScan VTI imaging platform and Micronucleus v3.0 BioApplication. Results from the analysis indicate that a single channel assay consisting of CHO cells stained with Acridine Orange leads to robust results and can be used as a fast end-point analysis assay for micronucleus formation as well as determination of dose dependent effectiveness of a compound. Three key advantages to using an HCS approach to micronucleus detection included:

1. A unique ability to simultaneously measure multiple biological events in individual cells within a population.

2. Increased productivity due to automation of a labor intensive manual assay.

3. Adaptability to both assay development and screening environments with flexibility in detection across several cell lines.

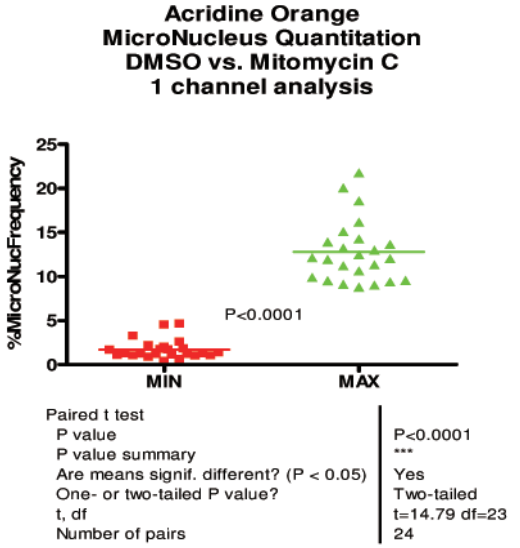

Figure 2. Minimum -vs- Maximum Response of mitomycin C
The percent of Micronuclei identified in 1000 binucleate cells with no treatment (red squares) and maximum treatment 200 ng/mL mitomycin C (green squares). The t-test indicated there was a significant significance between the two populations.

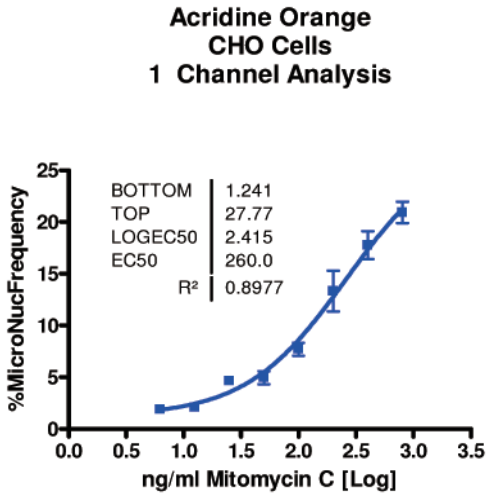

Figure 3. Dose Response of Mitomycin C
Eight point dose response of mitomycin C plotted against the percent of Micronuclei identified in 1000 binucleate cells. The EC_{50} was calculated at 260.0 ng/ML of mitomycin C.

Assay Configuration	Time per 96 well plate[1]	Number of wells per week.[2]
Single Channel, Acridine Orange	59 min	8640 wells

Figure 4. Throughput estimate based on one channel Acridine Orange assay with ArrayScan HCS Reader and Micronucleus BioApplication.

[1] Includes image acquisition, algorithm processing, and data transfer of 1000 targeted cells (binucleate) per well.

[2] Based on industry standard 18 hour instrument operating day. Calculated by processing eighteen 96 well plates per day during a five day work week.

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