Quantitative cell cycle analysis by using Countstar Rigel image cytometer

1. Introduction

Measuring the incorporation of DNA-binding dyes has been a well-established method for determining cellular DNA content in cell cycle analysis. Propidium iodide (PI) is a nuclear staining dye that is frequently applied in measuring cell cycle. In cell division, cells containing increased amounts of DNA display proportionally increased fluorescence. Differences in fluorescence intensity are used to determine the DNA content in each phase of the cell cycle. The Countstar Rigel system (Fig.1) is a smart, intuitive, multifunctional cell analysis instrument that can obtain precise data in cell cycle analysis and can detect cytotoxicity by cell viability assay. The easy-to-use, automated procedure guides you to complete a cellular assay from imaging and data acquisition.

Figure 1 Countstar Rigel system combines the functionalities of a digital microscope, an image cytometer and a cell counter in a single bench top instrument

2. Instruments and Materials:

Countstar Rigel
Countstar Chamber slider
Countstar Cell cycle kit
MCF7 cell line (ATCC)
Nocodazole (Sigma)

3. Methods

3.1 Cell growth medium

MCF7 cells were cultured in complete growth medium (DMEM, 2mM L-Glutamine, 1.5g/L Sodium bicarbonate and 10% FBS).

3.2 Drug treatment

(1). MCF7 cells were seeded in two 6-well plates at 300,000 cells/well in medium containing DMEM, 2mM L-Glutamine,1.5g/L Sodium bicarbonate and 10% FBS. Incubate the plates for 24h

at 37°C, 5% CO2 incubator.

(2). Replace old medium with seven different concentration of Nocodazolein fresh culture medium. The top concentration of Nocodazole was 400 nM, following with 2.5 folds serial dilution. Each sample has a two well. Leave two wells as DMSO control and keep the final concentration of DMSO same as samples. Incubator the plates at 37°C, 5% CO2 for a further 16 h.

3.3 Staining Procedures

(1) The cell number and cell viability detected by using "Trypan Blue Viability" assay.
(2) Prepare 0.3-0.5 million cells for cell cycle analysis according to the cell number in cell viability assay;
(3). Spin down cell sample at 400g for 3-5 minutes, remove medium, and then re-suspend cells in 200ul PBS;
(4). Remove supernatant and fix cells with 200ul 60% ethanol at 4℃ for at least 1 hour;
(5). Centrifuge at 400 g for 3-5 min and remove supernatant. Wash once with 200ul PBS;
(6). Re-suspend cells in 100ul cell cycle buffer containing1ul PI solution and 0.5ul RNAse A. Incubate for 30min at 37℃in the dark and then analyze samples by Countstar Rigel.
(7) The cell cycle program was created by setting Red channel to image PI fluorescence with 3 fields captured from each chamber. After analysis completed, data was exported and analyzed by FCS software.

Caution: PI is a potential carcinogen. It is recommended that the user wear protective clothing, gloves, and eye/face protection in order to avoid contact with skin and eyes.

4. Result

4.1 Assessing the cytotoxicity of Nocodazolein MCF7 cells

The cell viability assay is usually used in assessing anti-proliferative activity and cytotoxicity of compounds. The "Trypan Blue Viability" in Countstar Rigel was applied to measure the viability of different concentration of Nocodazole in MCF7 cells. The cytotoxicity of Nacodazole was accurately measured by the percentage of Sample viable cells/Control viable cells (Fig. 2). The IC50 of Nacodazole was evaluated by Graphpad prism 5 (Table. 1).

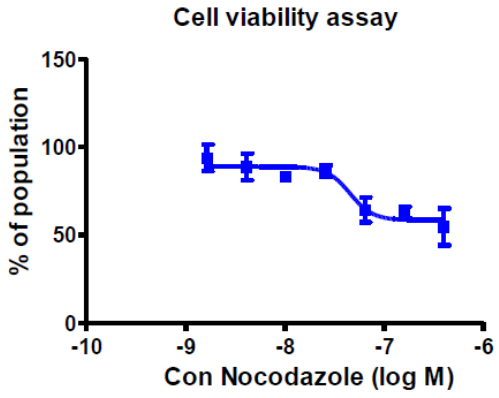


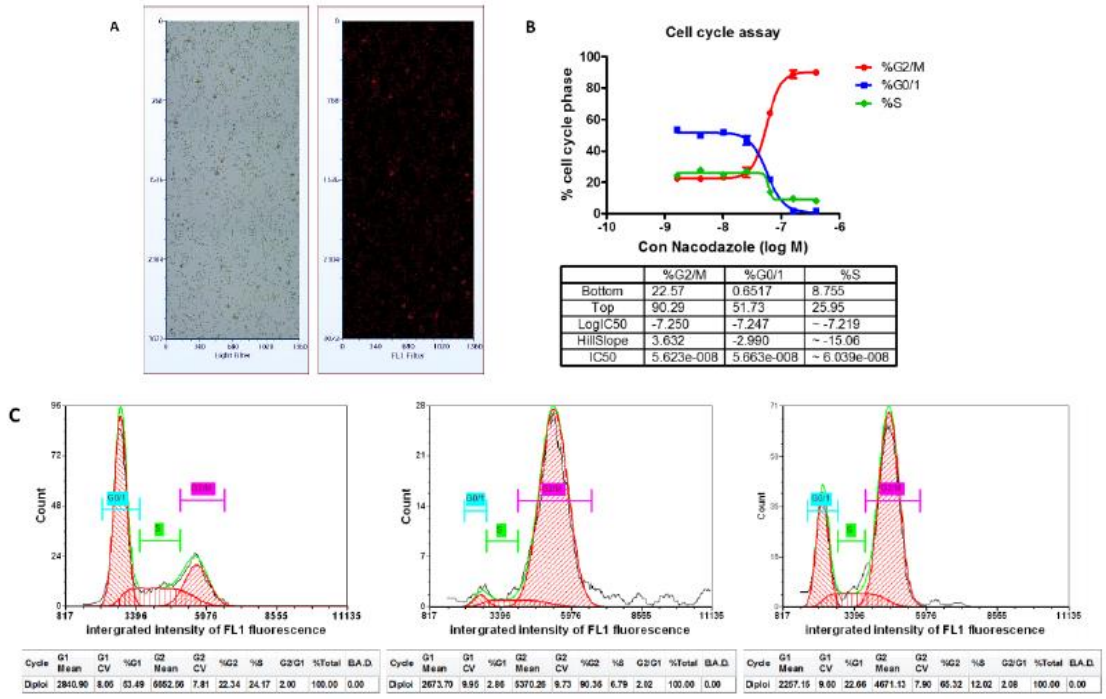
Figure 2 Viability of Cell of Different Concentration of Nacodazole

	Nocodazole
Bottom	58.56
Top	88.88
LogIC50	-7.344
HillSlope	-3.807
IC50	4.524e-008

Table 1 Cytotoxicity Analysis of Nacodazole

4.2 Quantitative cell cycle analysis with Countstar Rigel imaging system

The quantity of DNA in cell division can be predicted by determining the fluorescence intensity of PI which can intercalate with DNA. MCF7 cells were applied to analyze cell cycle treated by different concentration of Nocodazole. The bright field is used to identify individual cells and PI channel is applied to accurately count signal cells within clumps (Fig. 4A). The results were analyzed by FCS Express 5 image software and the IC50 of Nacodazole was evaluated by Graphpad prism 5.From Figure 4B, Nocodazole induced an obvious decrease in the G0/G1 phase and slightly reduction in S phase, while induced a sharp increase in the proportion of G2/M cells. The samples of DMSO control, 64nM and 400nM Nacodazole were chosen to present the intuitive results of cell cycle arresting in histogram (Fig. 4C).



	%G2/M	%G0/1	%S
Bottom	22.57	0.6517	8.755
Top	90.29	51.73	25.95
LogIC50	-7.250	-7.247	~ -7.219
HillSlope	3.632	-2.990	~ -15.06
IC50	5.623e-008	5.663e-008	~ 6.039e-008

Figure 3. The percent population of cells with or without Nacodazoletreatment in different cell cycle phase were displayed in population histogram and dose response curve. **A.** Images were obtained from PI channel and Bright field from Countstar Rigel. **B**. Percent population of cells indifferent cell cycle phase varied with different concentration of Nacodazole. **C**. Population histogram for the DMSO control, 64nM and 400nM Nocodazole samples generated by FCS Express 5 software.

5. Conclusion

The Countstar Rigel system is able to accurately analysis the dose response for cell cycle blockers by using a limited number of cells. FCS express software supplies similar function with FlowDrawto review percent population of cells in different cell cycle through the histogram. In addition, Countstar Rigel combines multiple functionalities and is a compact, application-driven, automated cell imaging system providing robust quantitative results by using preconfigured biological applications. Each pre-set assay is an easy-to-use, automated module that covers all steps of a specific biological assay to simplify routine cell laboratory tasks while providing high-quality scientific data. For quantifying cell cycle, Countstar Rigel supplies a sophisticated and reliable method.