Investigating cytotoxicity by using Countstar Rigel image cytometer

1. Introduction

Cytotoxicity assays are routinely used in many laboratories for a variety purposes from assessing the health of cell cultures to evaluating the toxicity of a panel of compounds. The measurement tool employed for these assays needs to be reliable, easy to use and relatively fast. The Countstar Rigel system (Fig 1) is a smart, intuitive cell analysis instrument that streamlines a wide variety of cellular assay including transfection, apoptosis, cell surface marker, cell viability and cell cycle assessments. The system provides robust fluorescence quantitative results. The easy-to-use, automated procedure guide you to complete a cellular assay form 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. Materials:

2.1 Countstar products

Countstar Rigel

Countstar Chamber slider

Countstar Apoptosis kit

2.2 Other materials

MCF-7 cell line (ATCC)

Camptothecin (Gene operation)

3. Methods:

3.1 Cell growth medium

MCF-7 cells were cultured in complete growth medium (DMEM, 2mM L-Glutamine, 1.5g/L sodium Bicarbonate and 10% FBS).

3.2 Drug treatment conditions

1. MCF-7 cells were added in 6-well plates at 200,000 cells/well in medium containing DMEM, 2mM L-Glutamine, 1.5g/L sodium Bicarbonate and 10% FBS 24 hr.
2. Add the drug with different amount (0μM -160μM) respectively (Fig.2) and incubated for 48 hr in 37°C 5% CO2 humidified incubator.
3. Harvest the cells.

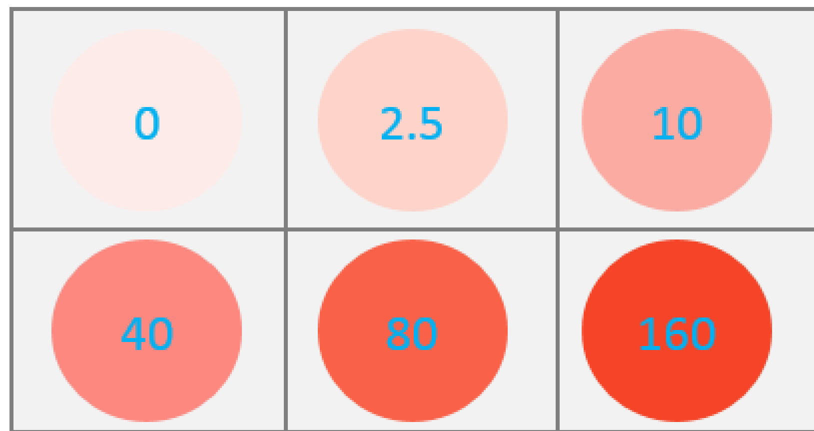


Figure 2. Signal well containing Camptothecin (0, 0.25, 10, 40, 80, 160 μM) are indicated.

3.3 Staining Procedure:

1. Prepare 0.3-0.5 million cells for next step;

2. Spin down cell sample at 400g for 3-5 minutes, aspirate medium, and then resuspend cells in 200μl PBS;

3. Mix the samples gently and spin down cell sample at 400g for 3-5 minutes, aspirate medium, and then resuspend cells in 100μl binding buffer;

4. Add 1.5μl of Annexin V-FITC;

5. Add 4μl of 7-AAD solution, gently pipette the cells up and down ten times;

6. Incubate for 10-15min at RT (25℃) in the dark;

7. Spin down cell sample at 400g for 3-5 minutes, aspirate medium, and the resuspend cells in 100μl binding buffer; analyze by Countstar Rigel

3.4 Imaging and analysis with Countstar Rigel

1. A dual-color application procedure was created by setting Green and Red channels to image FITC, 7-AAD fluorescence.
2. 3 fields were captured from each chamber, every sample was test for two times.
3. After imaging and initial analysis were complete, data were exported and analyzed by FCS software.

4. Result

4.1 Imaging and analysis with Countstar Rigel imaging system

A dual-color application procedure was created by setting Green and Red channels to image FITC, 7-AAD fluorescence, plus a bright field. Bright field picture reference segmentation was applied as a mask to sample the FITC fluorescence signal and 7-AAD. Example images of 0μM and 160μM are shown in Figure 3.

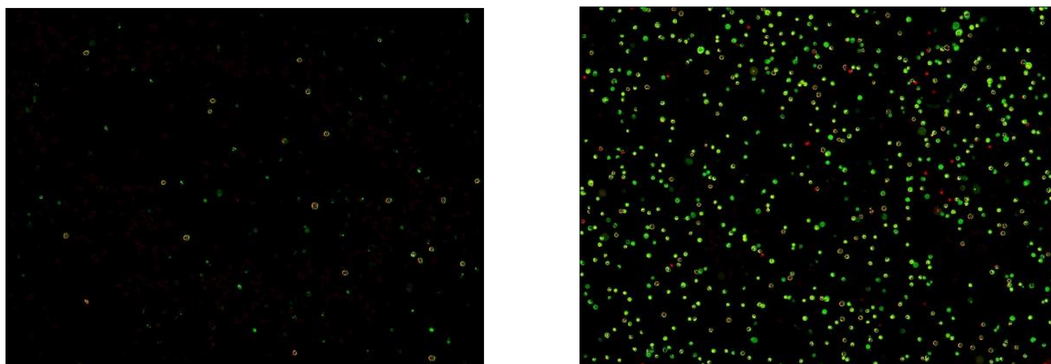
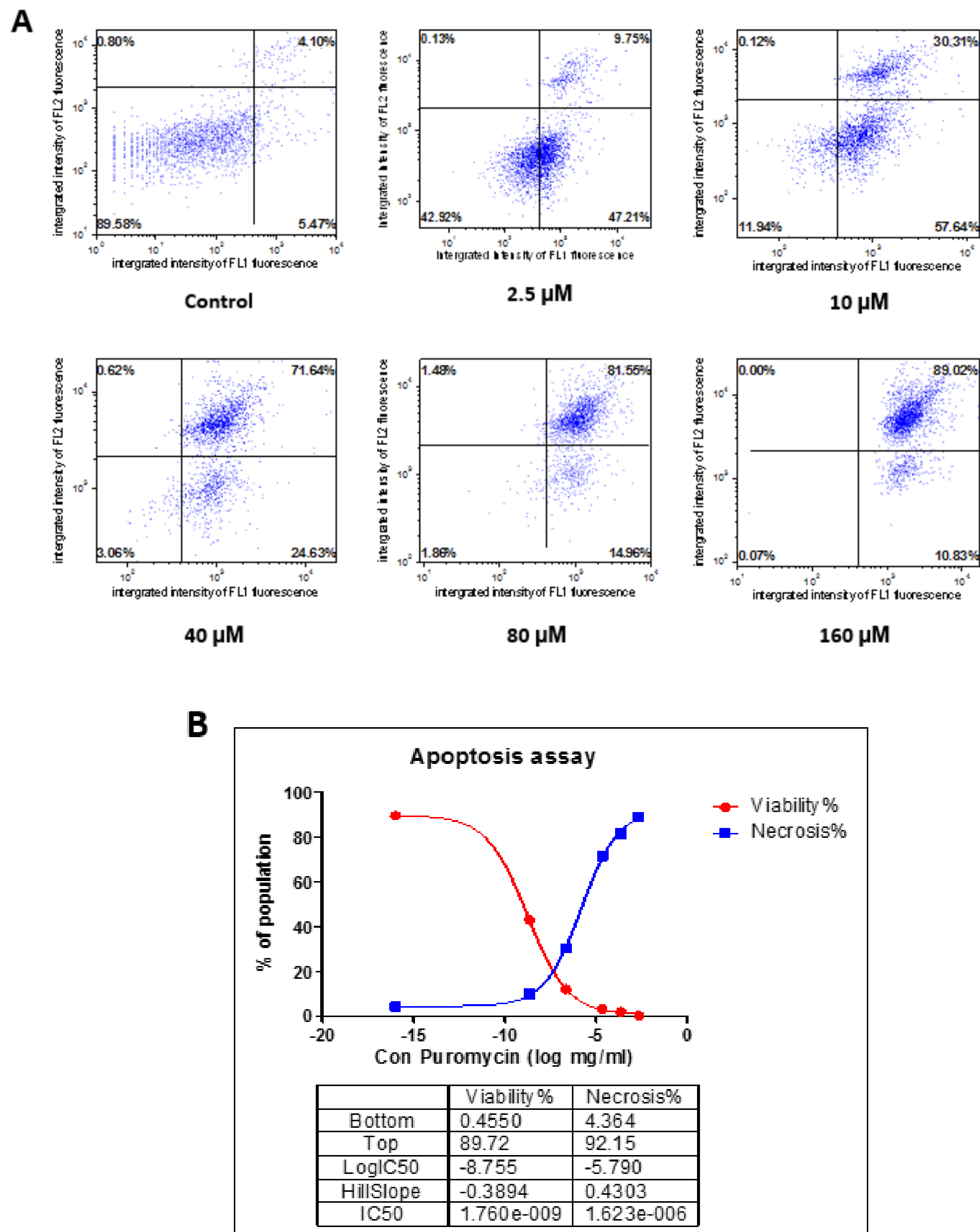


Figure 3. Images of 0μM and 160μM treated MCF-7 cells. **Left**. 0μM sample, show little fluorescence signal; representative image shown. **Right**. 160μM sample, most of the cells stained with both FITC and 7-AAD.

4.2 Kill curve for Camptothecin selection of MCF-7 cells

The results were analyzed by FCS5 image software, gating the cells dependent on the cell population and fluorescence intensity (Fig. 4A), the FCS express also supplies the function to review every signal cell, validation the data through the image (Fig. 4B). Graphpad prism5 was implemented to evaluate the IC50 of camptothecin.



	Viability %	Necrosis%
Bottom	0.4550	4.364
Top	89.72	92.15
LogIC50	-8.755	-5.790
HillSlope	-0.3894	0.4303
IC50	1.760e-009	1.623e-006

Figure 4. Kill curve assay showing identification of cell populations and results displayed as a scatter plot **A**. Images in dual channels were visually assessed to set a gating threshold to identify live, apoptotic, necrosis cell populations, and results were analyzed by FCS5 image software. **B**. Results indicating a more necrosis rate at increased levels of camptothecin.

5. Conclusion

The Countstar Rigel system provides a rapid and easy means of evaluating cytotoxicity. FCS express supplies the function to review every signal cell, validation the data through the image. In addition, it combines multiple functionalities and is a compact, application-driven, automated cell imaging system providing robust quantitative results through the use of 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. In addition, Countstar Rigel can provide quick answers to a cytotoxicity question prior to scaling up to a more extensive high throughput screening experiment.