Apoptosis and cell cycle analyses with the Quanta™ SC Flow System

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Abstract

Rapid and accurate apoptosis assay and cell cycle analysis are essential requirements in cancer research involving in vitro and in vivo studies. Cell death typically follows one of two distinct paths, apoptosis or necrosis. Apoptosis, or programmed cell death, is a physiological process by which unwanted cells are eliminated during development, normal cell turnover and tissue homeostasis. Oncogenesis is the results of uncontrolled cell division and alteration of apoptosis. Cells undergoing apoptosis display distinct morphological and biochemical changes such as translocation of phosphatidylserine (PS) from the inner side of the plasma membrane to the outer layer, damage of membrane structure integrity in the late stage apoptotic cells and activation of caspases.

In this report, a bench top flow cytometry system, Quanta SC that provides a rapid, accurate and cost effective way of measuring apoptosis and cell cycle is described. This system provides electronic volume, side scatter and 3 color fluorescence measurements with multiple excitation wavelengths at 366, 405, 435 or 488 nm from a Mercury (Hg) arc lamp or laser allowing flexible fluorochrome selection. Electronic volume measurement based on the Coulter Principle provides accurate cell size determination. Jurkat or K562 cells were treated with various reagents such as anti-CD95, tunicamycin, thapsigargin, colchicine and paclitaxel to induce apoptosis. Apoptotic cells were measured with various ways using the Quanta SC system. Externalization of PS to the cell surface was detected with Annexin-V-FITC. In combination with a cell impermeant dye, 7-aminoactinomycin D, live, early apoptotic and late apoptotic/dead cells were identified using the Quanta SC. Apoptotic cells were also detected with Alexa fluor 488-labeled anti-cleaved caspase 3 antibody after fixation and permeabilization. Cell cycle analysis was achieved by labeling DNA with 4′,6-diamidino-2-phenylindole (DAPI) or propidium iodide. G_0/G_1, S and G_2/M phase statistics plus cell size were accurately obtained by the Quanta SC. Together with easy-to-use software, the Quanta SC flow cytometry system provides a simple and flexible tool for apoptosis and cell cycle analysis on a daily basis.

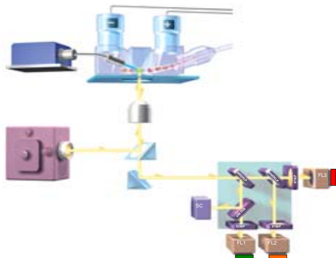
Note: For Research Use Only. Not for use in diagnostic procedures.

Introduction

System

- Syringe mechanism for precision enumeration
- Electronic volume for accurate sizing
- Three-color fluorescence detection
- Side scatter for granularity measurements
- Multicolor excitation for flexible fluorochrome selection
- Interchangeable filter block for optimum performance
- Cell Lab Quanta SC Software package
- Small footprint

Optical Path



Software

- Ease of use
- Collecting and analyzing data
- 10 histogram displays
- Fluorescent concentration (FC) and Fluorescent Surface Density (FSD) calculations
- Automated Compensation
- Create, share, and modify setup protocols
- Secure Login
- List mode data file
- Excel export file with selectable statistics and sample information

Materials and Methods

Reagents

- Nuclear Isolation Medium with DAPI (NIM-DAPI), PN 731085
- Anti-Cyclin A2-FITC, PN A22327
- FITC Conjugated isotopic control, PN IM0639
- Anti-Phospho-histone H3-Alexa Fluor 488, PN A24068
- Anti-Cleaved Caspase-3 (Asp175) – Alexa Fluor 488, PN A24067
- 7-Aminoactinomycin D (7-AAD), PN IM3422
- Anti-CD95, PN IM2387
- Flow-Check™ Fluorospheres, PN 6605359

Cell culture and treatment

Jurkat or K562 cells were cultured in RPMI-1640 supplement with 10% heat inactivated fetal bovine serum. Cells at a concentration of 1 x 10^6 cells/mL were treated with various reagents indicated.

Cell cycle analysis with NIM-DAPI

1. Transfer 500 ul of treated or control cells into a vial
2. Add 300 ul of NIM-DAPI solution.
3. Incubate for 0.5-2 min at room temperature
4. Analyze by Quanta SC with UV arc lamp.

Cell cycle analysis with anti-cyclin A2 or anti-phospho-histone H3

- Fixation and permeabilization
  1. Count cells using Vi-Cell®
  2. Aliquot 5 x 10^5 cells into a 1.5 or 2 mL assay tube.
  3. Collect cells by centrifugation and aspirate supernatant
  4. Wash cells with 1 mL of PBS (Invitrogen, PN14190-136).
  5. Fix and permeabilize cells by adding 1 mL ice-cold 100% methanol slowly to the cells while gently vortexing.
  6. Incubate 10 min on ice
  7. Collect cells in methanol by spinning at 300 *g* for 10 min at room temperature and aspirating supernatant.
- Staining
  1. Wash cells once with 1 mL of PBS + 0.5% BSA.
  2. Resuspend cells with 100 µL of PBS + 0.5% BSA.
  3. Add 20 µL of Anti-Cyclin A2-FITC, anti-phospho-histone H3-Alexa 488 or the isotypic control.
  4. Add 20 µL of 7-AAD
  5. Mix gently and incubate 60 min at 20-25°C in dark.
  6. Wash cells twice with 1.5 mL of PBS + 0.5% BSA.
  7. Analyze by Quanta SC.

Apoptosis analysis with Annexin V-FITC and 7-AAD

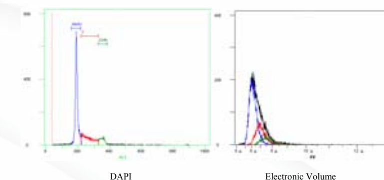
1. Pellet cells by centrifugation for 5 min at 300 g at 4°C
2. Resuspend cells in binding buffer at a concentration of 5-10 x 10^6 cells/mL
3. Aliquot 100 uL
4. Add 10 uL of Annexin V-FITC and 20 uL 7-AAD
5. Incubate 15 min on ice in dark
6. Analyze by Quanta SC within 30 min

Apoptosis analysis with anti-caspase 3-Alexa Fluor 488

- Fixation and permeabilization
  Follow same procedure described in the anti-cyclin A2 protocol.
- Staining
  1. Wash cells twice with 1 mL of PBS + 0.5% BSA.
  2. Resuspend cells with 90 µL of PBS + 0.5% BSA.
  3. Add 10 µL of Anti-Cleaved Caspase-3 (Asp175) – Alexa Fluor 488 or appropriate isotypic control.
  4. Incubate 60 min at 20-25°C in dark.
  5. Wash cells twice with 1 mL of PBS + 0.5% BSA.
  6. Resuspend cells with 0.5 mL PBS and analyze by Quanta SC.

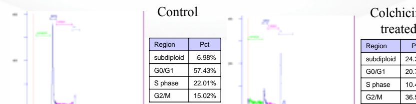
Results

Cell cycle analysis with NIM-DAPI (Jurkat cells)



Region	Diameter (µm)	MCV (µm³)	Count	Conc. (per ml x 1000)	Percentage of total	FL1 Mean	FL1 HPCV	FL1 CV	Color
G_0/G_1	6.22	125.7	10,901	304.2	62.21%	191.4	2.78%	4.86%	
S	7.06	183.9	4,313	120.3	24.61%	265.3	6.07%	11.71%	
G_2/M	7.58	228.4	1,664	46.5	9.50%	351.1	4.30%	3.79%	

Ultra-high resolution analysis of cell cycle with NIM-DAPI. The Quanta SC utilizes a Mercury arc lamp that produces an ideal 365 nm excitation line for DAPI. Statistics of each cell cycle phase were obtained with the Quanta SC software. Based on Coulter Principle of electronic volume measurement, the mean cell volume (MCV) and diameter were accurately measured with Quanta SC. Please note the mean volume of G_2/M cells (228.4 µm³) is almost twice the mean volume of G_0/G_1 cells (125.7 µm³) while only a 22% increase of diameter (7.58 µm vs. 6.22 µm) is observed (volume = $4/3\pi r^3$) indicating a clear advantage of electronic volume measurement. Because of the syringe mechanism, precise cell count and concentration (Con.) of each phase were also obtained.



Control

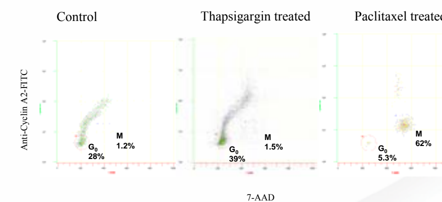
Region	Pct
subdiploid	6.98%
G0/G1	57.43%
S phase	22.01%
G2/M	15.02%

Colchicine treated

Region	Pct
subdiploid	24.27%
G0/G1	20.70%
S phase	10.49%
G2/M	36.56%

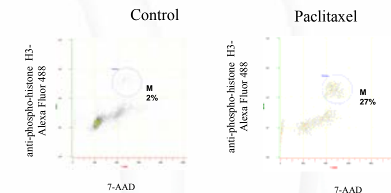
Control or colchicine (1 µM, 18 hours) treated Jurkat cells stained with NIM-DAPI. The analysis indicates a cell cycle arrest in the G2/M phase of colchicine treated cells.

Cell cycle analysis with anti-cyclin A2 (K562 cells)



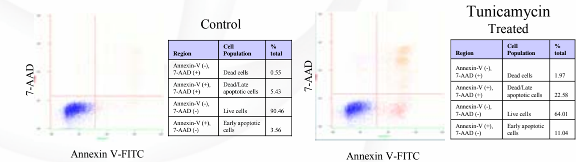
Control, thapsigargin (1 µM, 18 hours) or paclitaxel (0.5 µM, 18 hours) treated K562 cells stained with Anti-Cyclin A2-FITC and 7-AAD. The analyses indicate cell cycle arrests in the G_0 phase of thapsigargin treated cells and in the M phase of paclitaxel treated cells.

Cell cycle analysis with anti-phospho-histone H3 (Jurkat Cells)



Control or paclitaxel (0.5 µM, 18 hours) treated Jurkat cells stained with anti-phospho-histone H3-Alexa Fluor 488 and 7-AAD. The analysis indicate a cell cycle arrest in the M phase of paclitaxel treated cells.

Apoptosis analysis with Annexin V-FITC and 7-AAD (Jurkat cells)



Control

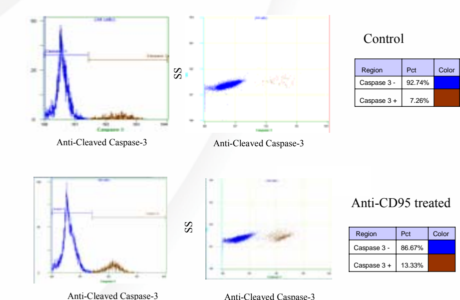
Region	Cell Population	% total
Annexin-V (-), 7-AAD (+)	Dead cells	0.55
Annexin-V (+), 7-AAD (+)	Dead/Late apoptotic cells	5.43
Annexin-V (-), 7-AAD (-)	Live cells	90.46
Annexin-V (+), 7-AAD (-)	Early apoptotic cells	3.56

Tunicamycin Treated

Region	Cell Population	% total
Annexin-V (-), 7-AAD (+)	Dead cells	1.97
Annexin-V (+), 7-AAD (+)	Dead/Late apoptotic cells	22.58
Annexin-V (-), 7-AAD (-)	Live cells	64.01
Annexin-V (+), 7-AAD (-)	Early apoptotic cells	11.04

Control or tunicamycin (1 µg/mL, 18 hours) treated Jurkat cells stained with Annexin V-FITC and 7-AAD .

Apoptosis analysis with anti-caspase 3-Alexa Fluor 488 (Jurkat cells)



Control

Region	Pct	Color
Caspase 3 -	92.74%	
Caspase 3 +	7.26%	

Anti-CD95 treated

Region	Pct	Color
Caspase 3 -	86.67%	
Caspase 3 +	13.33%	

Control and Anti-CD95 (100 ng/mL, 4 hours) treated Jurkat cells stained with Anti-Cleaved Caspase-3- Alexa Fluor 488.

Conclusion

Together with Beckman Coulter's reagent offering and powerful easy-to-use software, the Quanta SC flow cytometry system provides a simple, flexible and high performance tool for apoptosis and cell cycle analysis on a daily basis.