
Fast, Accurate Apoptosis and Cell Cycle Studies Using Kits and Templates on the BD Accuri™ C6

Presented by:

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Outline

- Background: Apoptosis and Cell Cycle
- Introduction to the BD Accuri™ C6 Flow Cytometer
- BD Kits and Templates
- BD Templates on the Web
- How to Create Your Own Templates
- Coming Soon....



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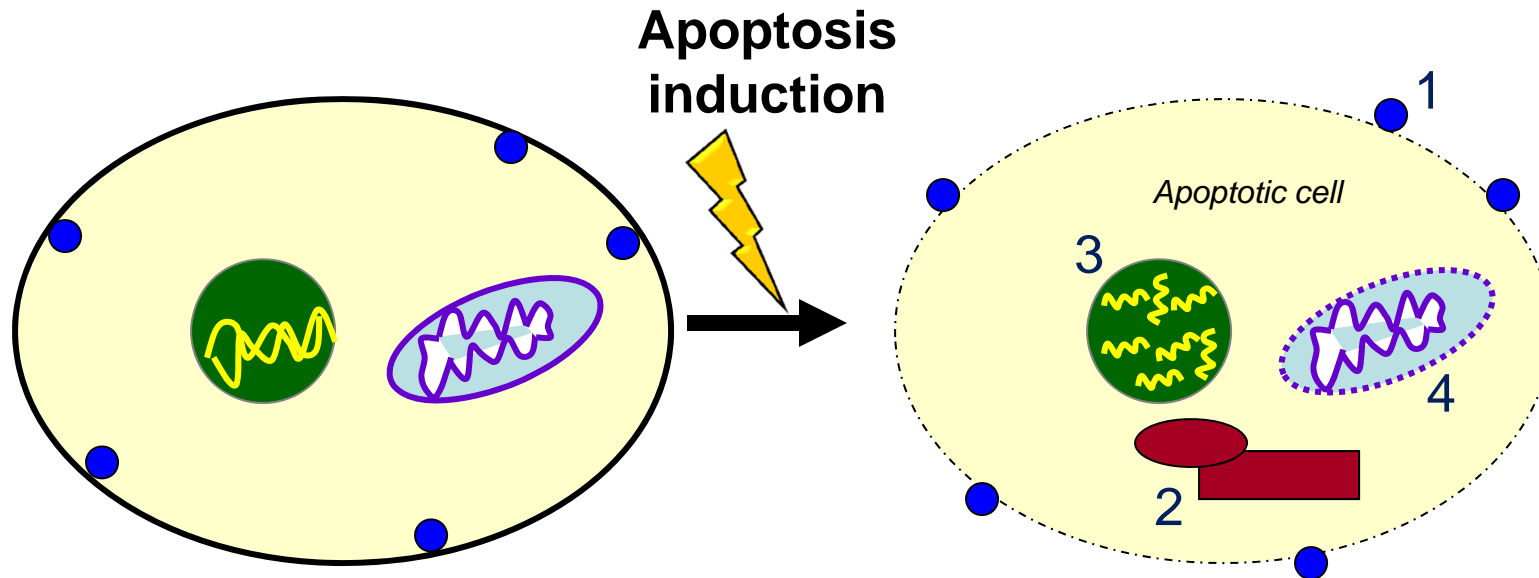
Apoptosis

- Definition:
 - The process leading to controlled self destruction of a cell. Cells undergo death neatly without damaging their neighbors. Apoptosis is a “programmed event.”
- Importance of Apoptosis
 - Cell termination
 - Viral infection, cancer
 - Homeostasis
 - Tumor, diseases
 - Development
 - Organs, appendages, patterning
 - Lymphocyte development
 - Thymic selection
 - Drug discovery studies



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Apoptosis at a Glance



1. Phosphotidyl serine (PS) exposed
2. Mitochondrial potential decreases
3. Caspases activated
4. DNA fragmentation

Summary of Apoptosis Assays

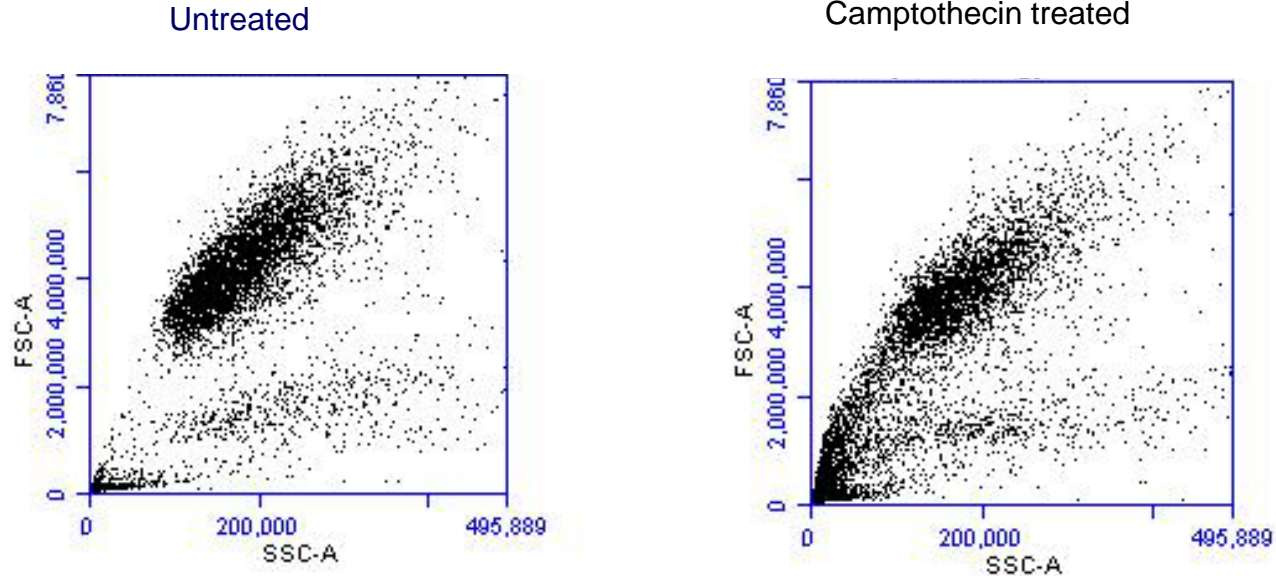
Feature Measured	Assay	Key Benefits
Plasma Membrane Alterations	Annexin V Binding Assay: <ul style="list-style-type: none">• Single Conjugates• Annexin V Kits	<ul style="list-style-type: none">• Detects early apoptosis• Quick and easy
Mitochondrial Changes	<ul style="list-style-type: none">• BD™ MitoScreen	<ul style="list-style-type: none">• Quick and easy
Caspase Activation	<ul style="list-style-type: none">• Active Caspase-3 Flow Kit	<ul style="list-style-type: none">• Specific antibodies can detect activated vs uncleaved caspase-3• Can be multiplexed
DNA Fragmentation	<ul style="list-style-type: none">• APO-BrdU TUNEL Assay• APO-Direct TUNEL Assay	<ul style="list-style-type: none">• Works with adherent cells



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Apoptosis: Scatter Properties

Cell shrinkage during apoptosis is associated with a decrease in forward scatter. Analysis of light scatter often is combined with other assays.



Formation of apoptotic vesicles

- **Increases side scatter**

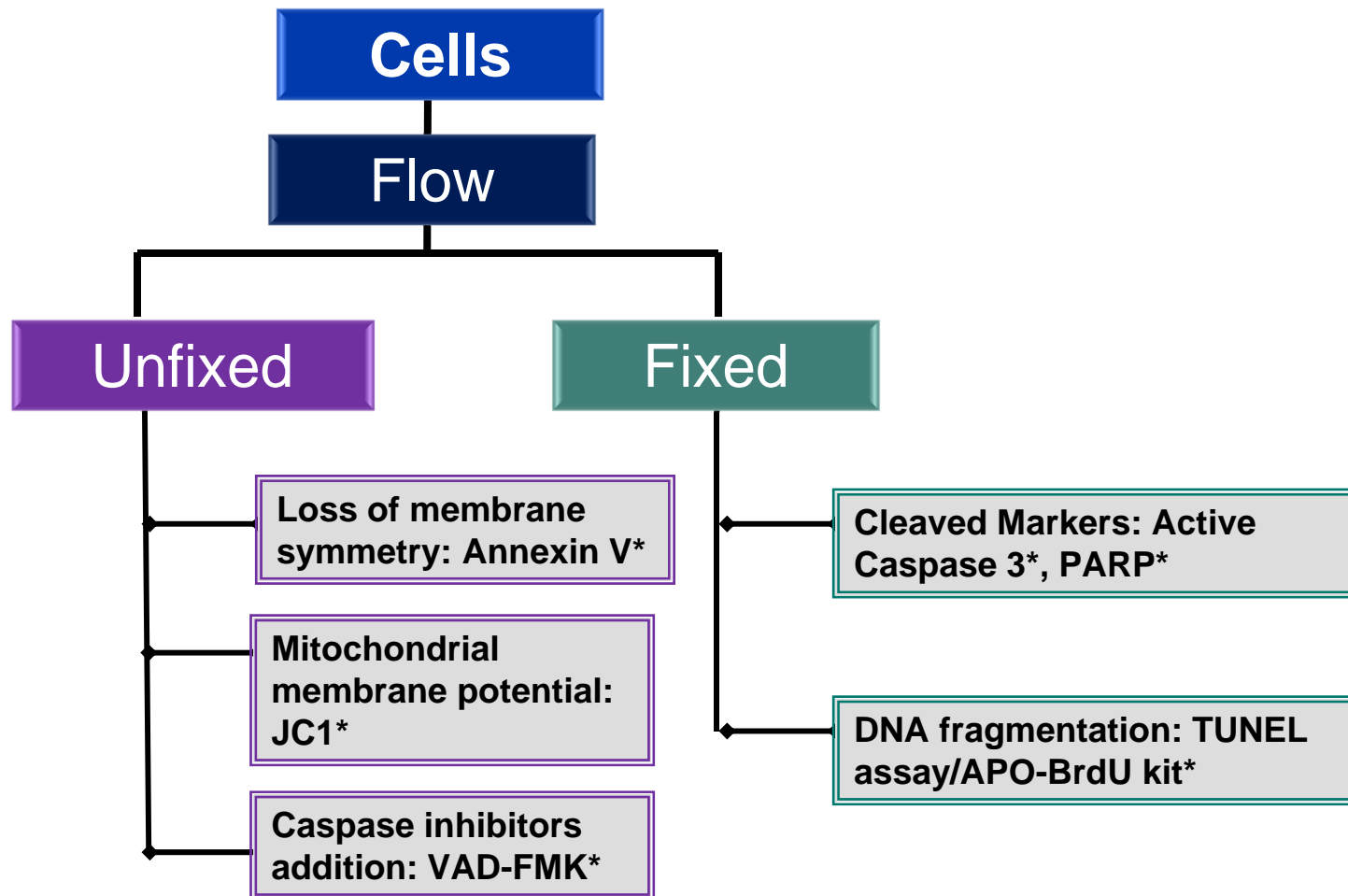
Reduced refractive index of apoptotic cells

- **Decreases forward scatter**



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Apoptosis Method Decision Tree

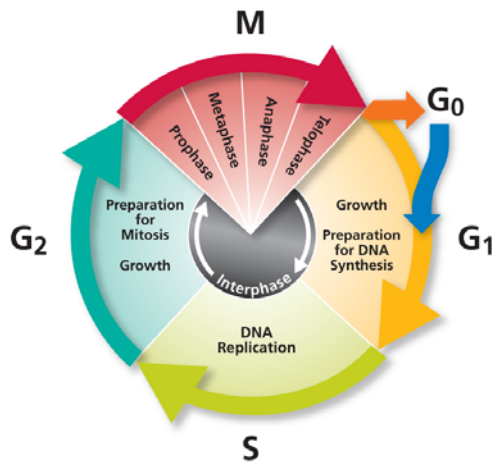


* BD products available



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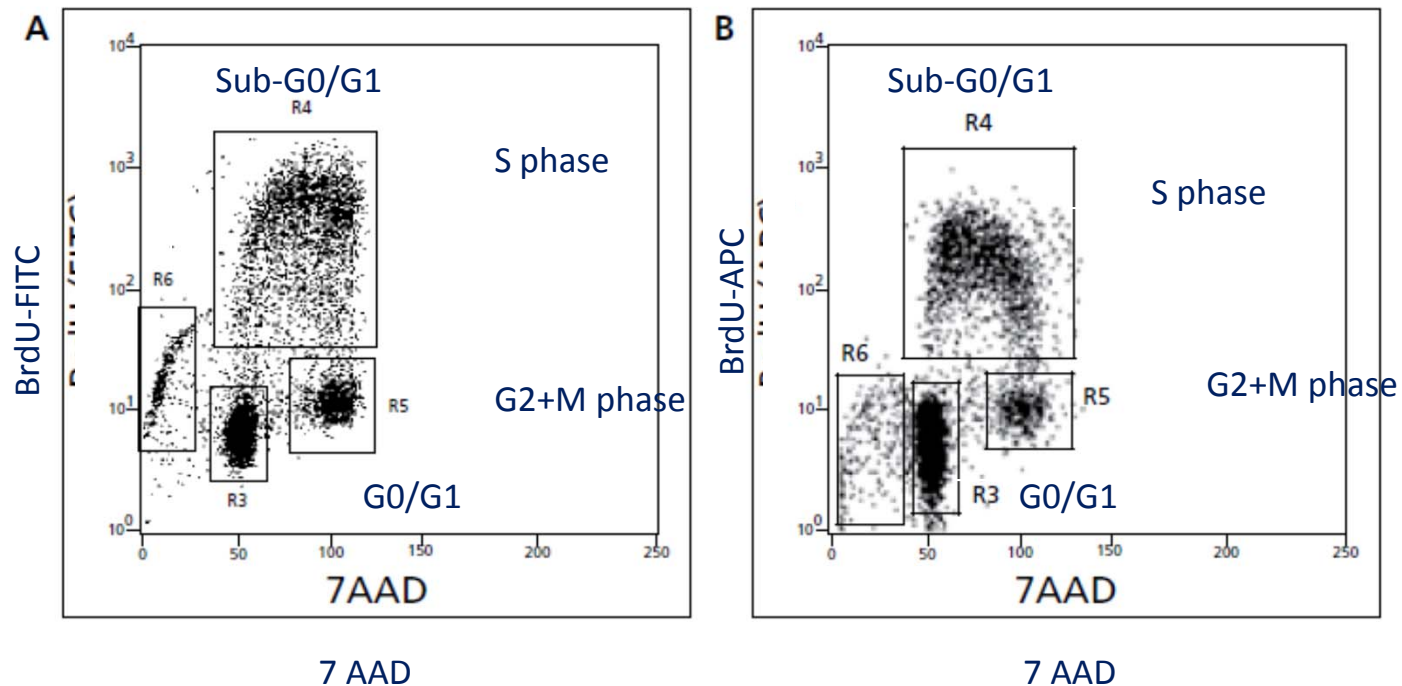
Cell Cycle and Proliferation Analysis



- There are several methods available to measure cell cycle and proliferation.
- Flow cytometry allows quantitative detection of results.

Measures	Reagents	Mechanism	Technology	Sample Types
DNA	Propidium Iodide (PI), 7-aminoactinomycin D (7-AAD)	Interaction into DNA double strands	Flow cytometry	Fixed, permeabilized, and for live/dead discrimination in intact cells
Cell Dyes	BD Horizon™ violet proliferation dye 450 (VPD450)	Diffuses into live cells and is hydrolyzed by intracellular non-specific esterases to become fluorescent products.	Flow cytometry	Live proliferating cells
Newly Synthesized DNA	BrdU and antibodies to BrdU	Bromodeoxyuridine replaces thymidine (T) in dividing DNA. It is then detected by antibodies to BrdU.	Flow cytometry, cell imaging, immunohistochemistry	Fixed and permeabilized cells, treated tissues (cell imaging, immunohistochemistry only)
Protein Level	Antibodies to Ki67, PCNA	Levels increase as a result of proliferation.	Flow cytometry, bioimaging, immunohistochemistry, Western blot	Fixed cells, tissues, and extracts
Protein Level	Antibodies to cyclins, retinoblastoma (Rb), other cell cycle markers	Levels go up and down at different stages of the cell cycle.	Flow cytometry, bioimaging, immunohistochemistry, Western blot	Fixed cells, tissues, and extracts
Protein Modification	Antibodies to phosphorylated histone H3, cyclin dependent kinases (cdk)	Proteins become phosphorylated as a result of proliferation or changes to the cell cycle.	Flow cytometry, bioimaging, immunohistochemistry, Western blot	
BD™ CBA (for quantitative detection)	Fixed cells, tissues, and extracts			

Cell Cycle Analysis of a Population Stained for Incorporated BrdU and Total DNA Levels (7-AAD)



Human peripheral blood mononuclear cells (PBMCs) were stimulated with anti-CD3/CD28 for 48 hours and re-stimulated with PMA+Ionomycin for 4 hours, and BrdU was added for the final 1 hour. Cells were then harvested and stained using the BrdU staining protocol.



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- Background: Apoptosis
- **Introduction to the BD Accuri™ C6 Flow Cytometer**
- BD kits and templates
- BD templates on the web
- How to create your own templates
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Introduction

The BD Accuri™ C6 Flow Cytometer System



An Affordable, Full-Featured, Easy-to-Use Flow Cytometer

Two lasers and six detectors



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Intuitive Software

The screenshot shows the BD CSampler software interface with various components annotated:

- Sample Grid:** A 12x12 grid of wells (A1-A12, D1-D12, E1-E12, F1-F12, G1-G12, H1-H12) with a color-coded status for each well.
- Cytometer Status:** A green indicator showing 'C6 is connected and ready.'
- Fluidics Controls:** Settings for Run (Unlimited/Limits), Fluidics (Slow/Medium/Fast), and Threshold (Set Threshold).
- Run Criteria:** Buttons for Backflush, Unclog, Wash, and Agitate.
- Real-Time Updates:** A section showing Last Run and Cumulative statistics (Events, Time, Microliters, Events/Sec, Events/μL).
- Plot Statistics:** A table summarizing data for various plots, including Count, Volume (μL), % of This Plot, % of All, and Mean/ CV/ Median values for different parameters.



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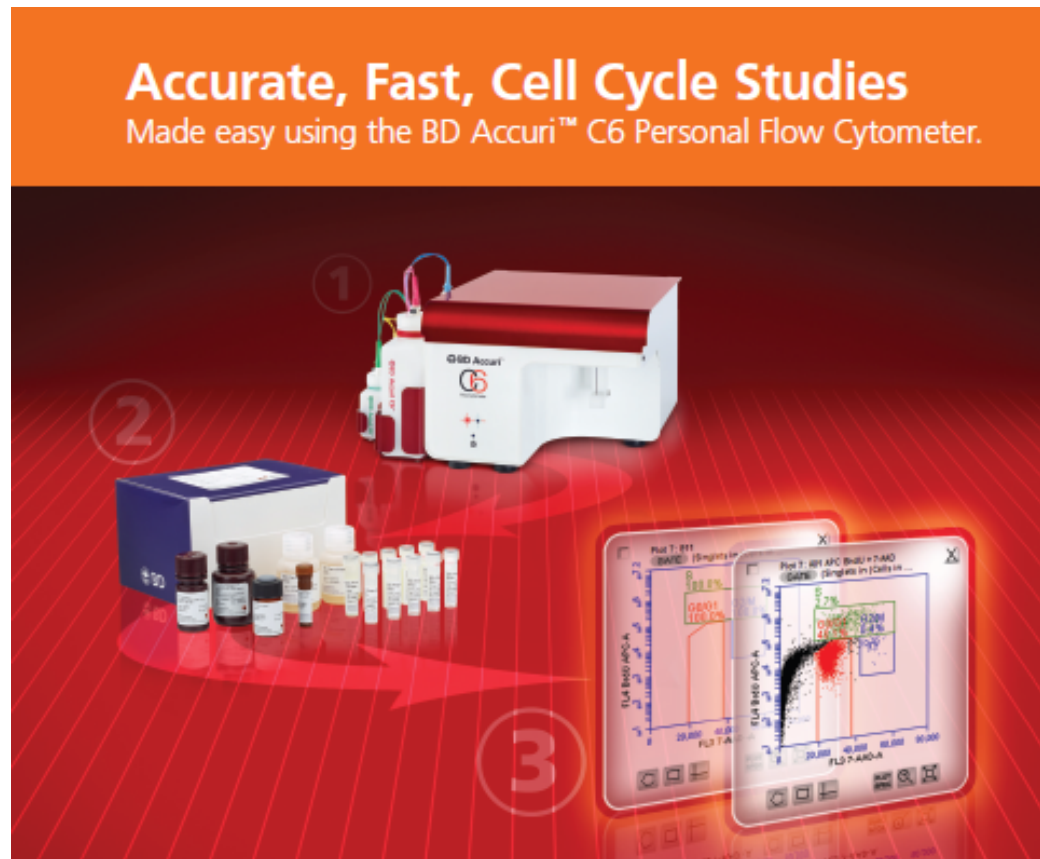


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BD Kits and Templates

Accurate, Fast, Cell Cycle Studies

Made easy using the BD Accuri™ C6 Personal Flow Cytometer.



Arguably, as easy as cell analysis is going to get.



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BD Cell Biology Kits

Application	Kit	Cat. No.
Apoptosis	Annexin V FITC Apoptosis Detection Kit II	<u>556570</u>
	Annexin V PE Apoptosis Detection Kit I	<u>559763</u>
	MitoScreen (JC-1) Kit	<u>551302</u>
	Caspase-3 PE Assay Kit	<u>550914</u>
Cell Cycle and DNA	DNA Reagent Kit	<u>340242</u>
	FITC BrdU Flow Kit	<u>559619</u>
	APC BrdU Flow Kit	<u>552598</u>



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What is a Template?

The screenshot displays the BD Accuri C6 software interface with a pre-defined workspace template. The interface is organized into several sections:

- Grid:** A 12x8 grid of sample wells labeled A1 through H12. Well H12 is highlighted in red.
- Run Settings:** Includes options for 'Run Unlimited' or 'Run with Limits', 'Fluidics' settings (Slow, Medium, Fast), and 'Threshold' settings.
- Plots:** Three plots are visible:
 - Plot 1: H12 (GATE):** Shows a single population (P1) at 100.0%.
 - Plot 2: H12 (GATE) (P1 in all):** Shows a population (P1) at 100.0%, with sub-populations: Q1-UL (100.0%), Dead (100.0%), Live (100.0%), and Apoptotic (100.0%).
 - Plot 3: H12 (GATE) (P1 in all):** Shows a population (P1) at 100.0%, with sub-populations: Q5-UL (100.0%), Dead (100.0%), Live (100.0%), and Apoptotic (100.0%).
- Statistics:** A table at the bottom provides statistical data for each plot, including Count, Volume (µL), % of This Plot, % of All, Mean FSC-A, Mean SSC-A, CV FSC-A, CV SSC-A, Median FSC-A, and Median SSC-A.

A BD Accuri™ template is a pre-defined workspace that includes gates, labels, run criteria, and compensation settings for a specific assay.



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Pre-Defined Gating and Run Criteria for Ease of Use

The screenshot displays the BD Accuri C6 software interface, which is divided into several functional areas:

- Collect Panel:** Shows a 12x8 grid of sample wells (A12-H12). Well H12 is highlighted with a red border and a blue arrow pointing to it.
- Run Settings Panel:** Contains controls for 'Run Unlimited', 'Run with Limits', 'Flow Rate' (14 µL/min), 'Core Size' (10 µm), and a 'Threshold' set to 80,000 on FSC-H.
- Statistics Panel:** Displays three plots:
 - Plot 1: H12 (GATE) [No Gating]**: A scatter plot of SSC-A vs FSC-A with a red dashed gate labeled 'P1 100.0%'.
 - Plot 7: H12 (GATE) [P1 in all]**: A plot of FL3 Propidium Iodide-A vs FL1 Annexin V FITC-A, showing a 2x2 quadrant with 'Q1-UL 100.0%' and 'Dead 100.0%'.
 - Plot 9: H12 (GATE) [P1 in all]**: A plot of FL3 Propidium Iodide-A vs FL1 Annexin V FITC-A, showing a 2x2 quadrant with 'Live 100.0%' and 'Apoptotic 100.0%'.
- Bottom Panel:** Includes a 'RUN' button, 'Last Run' statistics, and a table of data for the selected plots.

Plot 1: H12	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	0	0	100.00%	100.00%	0.00	0.00	0.00%	0.00%		
P1	0	0	100.00%	100.00%	0.00	0.00	0.00%	0.00%		

Plot 7: H12 Gated on (P1 in all)	Count	Volume (µL)	% of This Plot	% of All	Mean FL1 Annexin V FITC-A	Mean FL3 Propidium Iodide-A	CV FL1 Annexin V
This Plot	0	0	100.00%	100.00%		0.00	0.00
Q1-UL	0	0	100.00%	100.00%		0.00	0.00
Dead	0	0	100.00%	100.00%		0.00	0.00
Live	0	0	100.00%	100.00%		0.00	0.00
Apoptotic	0	0	100.00%	100.00%		0.00	0.00

Plot 9: H12	Count	Volume (µL)	% of This Plot	% of All	Mean FL1 Annexin V FITC-A	Mean FL3 Propidium Iodide-A	CV FL1 Annexin V
This Plot	0	0	100.00%	100.00%		0.00	0.00
Live	0	0	100.00%	100.00%		0.00	0.00
Apoptotic	0	0	100.00%	100.00%		0.00	0.00



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Compensation

The screenshot displays the BD Accuri C6 software interface. The top navigation bar includes 'Collect', 'Analyze', 'Statistics', and 'Batch Analysis'. The 'Collect' tab is active, showing a 12x12 grid of sample locations (A1-H12) with A1 selected. Below the grid are 'Run Settings' (Run Unlimited/with Limits, Fluidics: Slow/Medium/Fast, Threshold) and a 'RUN' button. A 'Compensation Settings for A01' dialog box is open, showing options to correct FL1-FL4 by subtracting a percentage of other channels. The dialog includes a vertical slider for compensation adjustment.

Three plots are visible in the background:

- Plot 1: A01 (GATE) [No Gating]
- Plot 8: A01 (GATE) (P1 in all)
- Plot 11: A01 (GATE) (P1 in all)

Below the plots are data tables for Plot 1: A01, Plot 8: A01, and Plot 11: A01.

Plot 1: A01	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	0	0	100.00%	100.00%	0.00	0.00	0.00%	0.00%		
P1	0	0	100.00%	100.00%	0.00	0.00	0.00%	0.00%		

Plot 8: A01	Count	Volume (µL)	% of This Plot	% of All	Mean FL2 Annexin V PE-A	Mean FL3 7-AAD-A	CV FL2 Annexin V PE-A	CV FL3 7-AAD-A
This Plot	0	0	100.00%	100.00%	0.00	0.00	0.00%	0.00%
Q2-UL	0	0	100.00%	100.00%	0.00	0.00	0.00%	0.00%
Dead	0	0	100.00%	100.00%	0.00	0.00	0.00%	0.00%
Live	0	0	100.00%	100.00%	0.00	0.00	0.00%	0.00%
Apoptotic	0	0	100.00%	100.00%	0.00	0.00	0.00%	0.00%

Plot 11: A01	Count	Volume (µL)	% of This Plot	% of All	Mean FL2 Annexin V PE-A	Mean FL3 7-AAD-A	CV FL2 Annexin V PE-A	CV FL3 7-AAD-A
This Plot	0	0	100.00%	100.00%	0.00	0.00	0.00%	0.00%
Q2-UL	0	0	100.00%	100.00%	0.00	0.00	0.00%	0.00%
Dead	0	0	100.00%	100.00%	0.00	0.00	0.00%	0.00%
Live	0	0	100.00%	100.00%	0.00	0.00	0.00%	0.00%
Apoptotic	0	0	100.00%	100.00%	0.00	0.00	0.00%	0.00%



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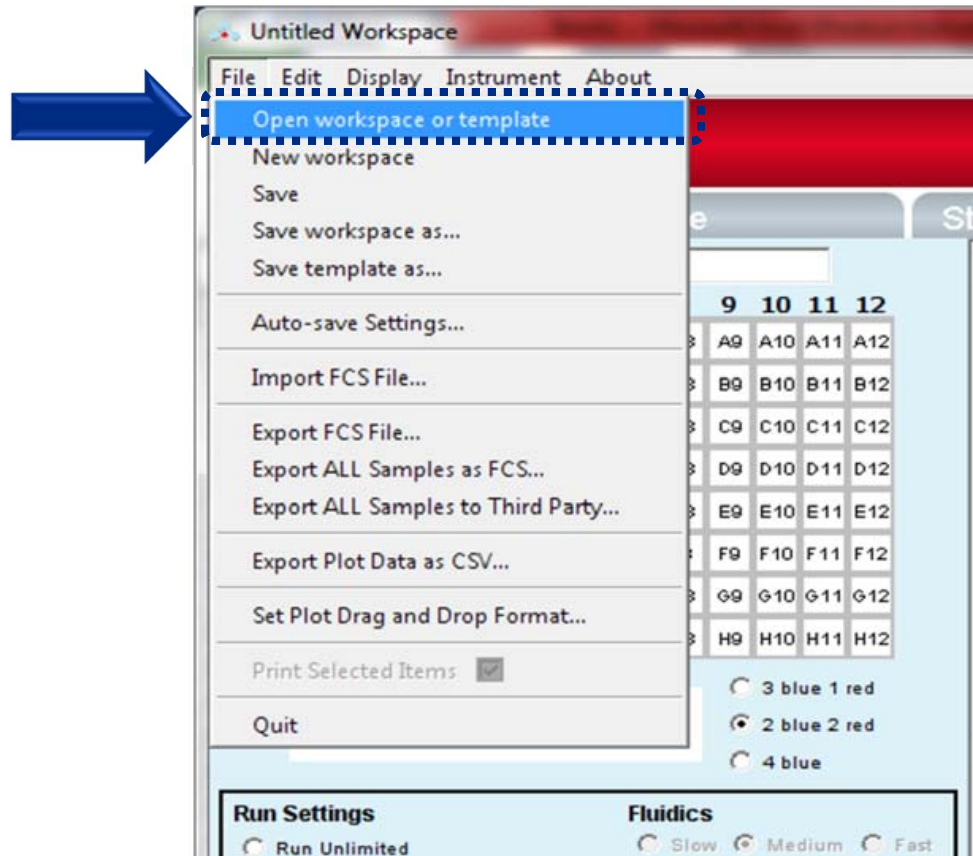
Where to Find the Templates

- www.bdbiosciences.com/go/templates



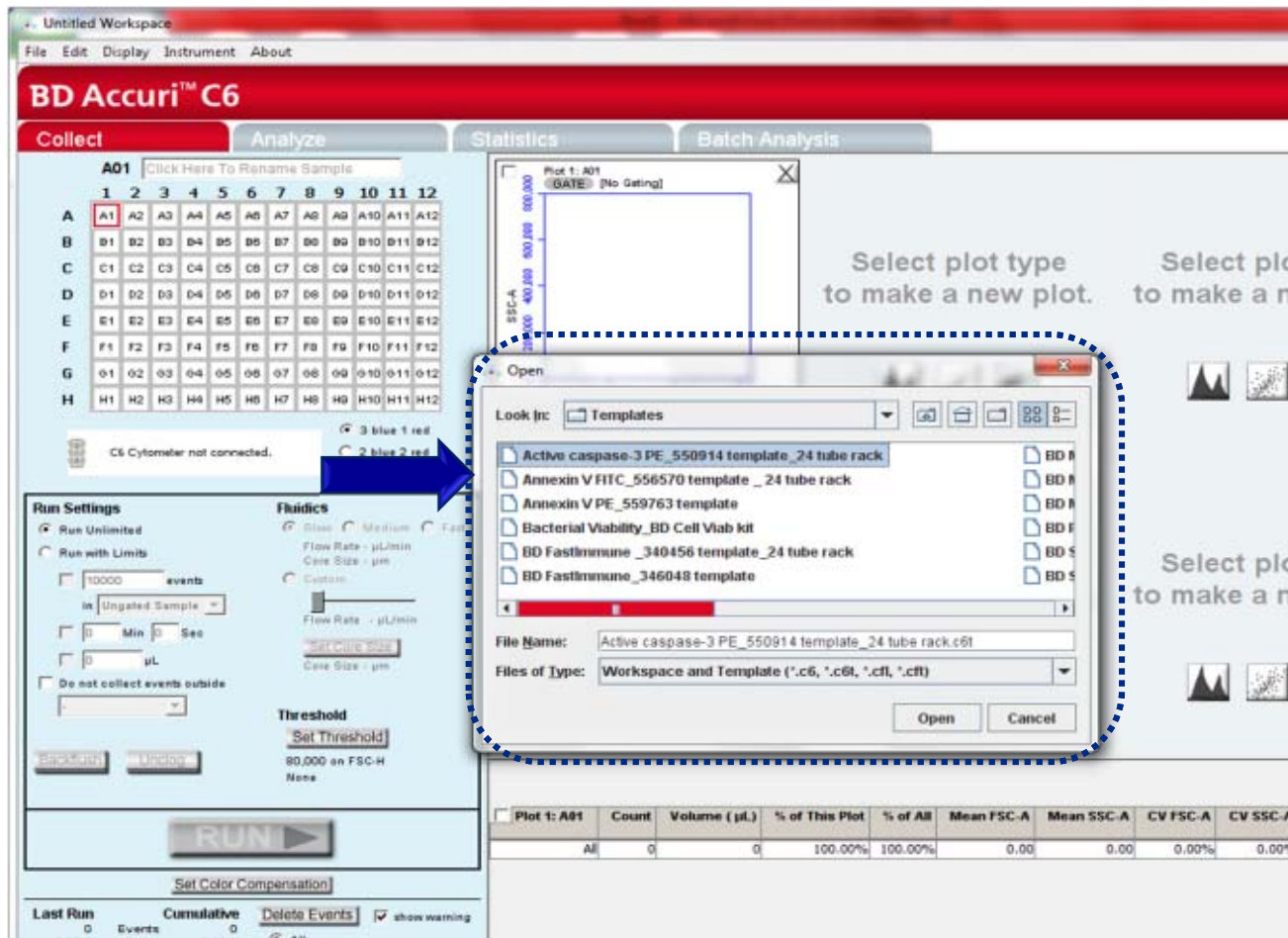
Category	Product Information Sheet	Brand	Kit	Cat. No.	Template
Cell Biology	BD Apoptosis Kits and Templates	BD Pharmingen™	Annexin V FITC Apoptosis Detection Kit II	556570	Download
		BD Pharmingen™	Annexin V PE Apoptosis Detection Kit I	559763	Download
		BD™	MitoScreen (JC-1) Kit	551302	Download
		BD Pharmingen™	Caspase-3 PE Assay Kit	550914	Download
	BD Cell Cycle and DNA Kits and Templates	BD Cycletest™ Plus	DNA Reagent Kit	340242	Download
		BD Pharmingen™	FITC BrdU Flow Kit	559619	Download
		BD Pharmingen™	APC BrdU Flow Kit	552598	Download
Immunology	BD Naïve/Memory T-Cell Kits and Templates	BD Multitest™	CD45RA/CD45RO/CD3/CD4	340571	Download
		BD Multitest™	CD45RA/CD62L/CD3/CD4	340977	Download
		BD Pharmingen™	Human Naïve/Memory T Cell Panel	561438	Download
	BD Intracellular T-Cell Kits and templates	BD Pharmingen™	Th1/Th2/Th17 Phenotyping Kit	560751	Download
		BD FastImmune™	IFN-γ/CD69/CD8/CD3 Kit	346048	Download
		BD FastImmune™	IFN-γ/IL4 Kit	340456	Download

How to Open a Template



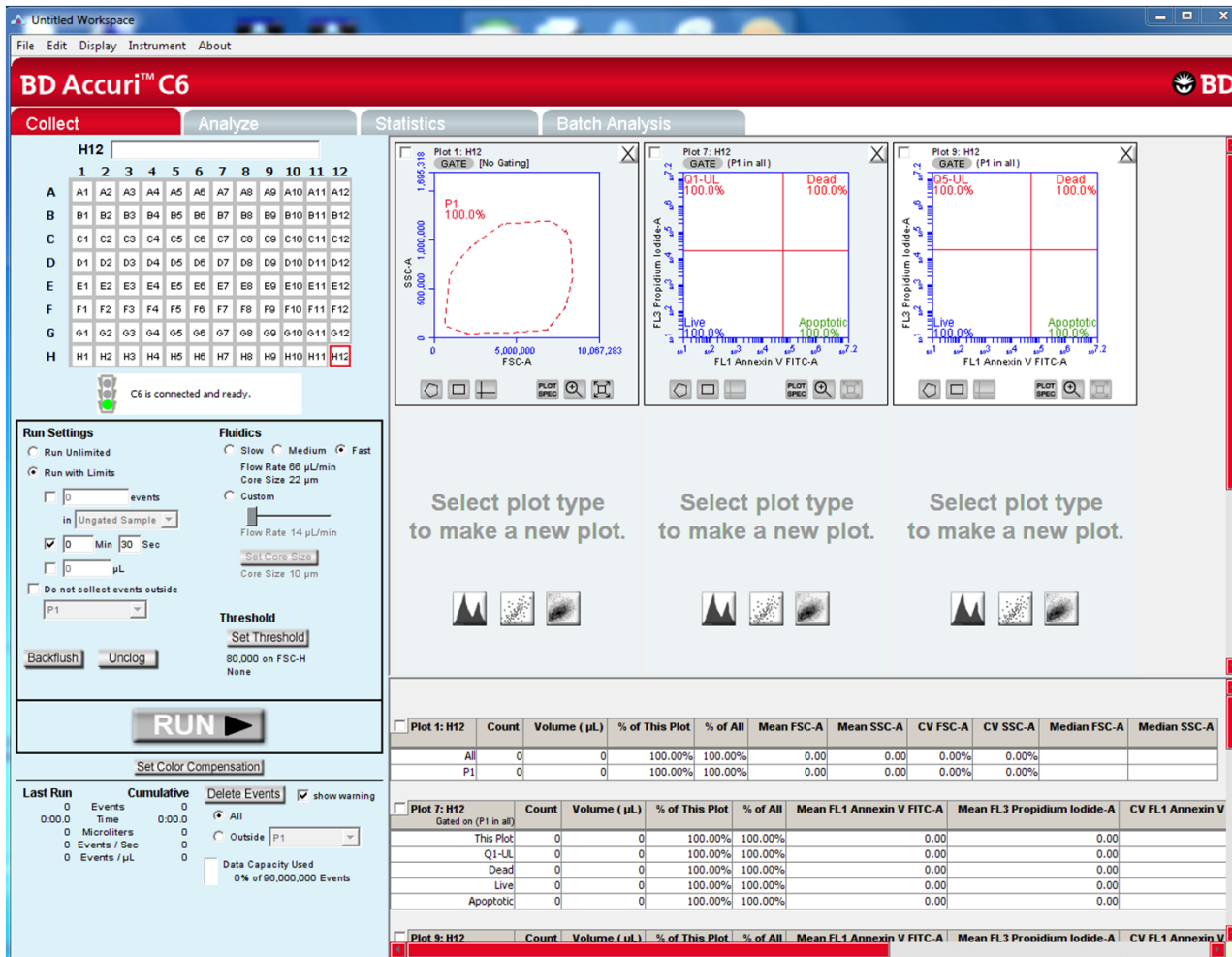
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How to Open a Template



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How to Use a Template: Example Using the Annexin V Apoptosis Detection Kit (Cat. No. 556570)



Reagents:
Annexin V FITC
Propidium Iodide



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How to Use a Template:

Verify Settings, Collect, Adjust as Necessary

The screenshot displays the BD Accuri C6 software interface. The top menu bar includes 'File', 'Edit', 'Display', 'Instrument', and 'About'. The main workspace is titled 'Untitled Workspace' and contains three plots:

- Plot 1: A01 DMSO FITC + PI (GATE: [No Gating])**: Shows a scatter plot of SSC-A vs FSC-A with a gate labeled 'P1' at 79.0%.
- Plot 7: A01 DMSO FITC + PI (GATE: (P1 in all))**: Shows a scatter plot of FL3 Propidium Iodide-A vs FL1 Annexin V FITC-A with gates for 'Live' (40.0%), 'Apoptotic' (8%), and 'Dead' (2.1%).
- Plot 9: A01 DMSO FITC + PI (GATE: (P1 in all))**: Shows a scatter plot of FL3 Propidium Iodide-A vs FL1 Annexin V FITC-A with gates for 'Live' (40.0%), 'Apoptotic' (8%), and 'Dead' (2.1%).

Below the plots, there are three prompts: 'Select plot type to make a new plot.' with corresponding plot icons.

The bottom right corner features a statistics table for Plot 9:

	Count	Volume (µL)	% of This Plot	% of All	Mean FL1 Annexin V FITC-A	Mean FL3 Propidium Iodide-A	CV FL1 Ar
This Plot	4,621	33	100.00%	79.02%	79,522.95	17,582.75	
Q5-UL	0	33	0.00%	0.00%	0.00	0.00	
Dead	99	33	2.14%	1.69%	2,482,063.11	778,837.24	
Live	1,848	33	39.99%	31.60%	4,221.66	788.16	
Apoptotic	2,674	33	57.87%	45.73%	42,613.95	1,005.42	

On the left side, the 'Run Settings' panel is visible, showing options for 'Run Unlimited' or 'Run with Limits', 'Fluidics' settings (Flow Rate, Core Size), and 'Threshold' settings. A 'RUN' button is prominently displayed.



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Ready to Acquire Data

BD Accuri™ C6

Collect Analyze Statistics Batch Analysis

A01 DMSO FITC + PI

1	2	3	4	5	6	7	8	9	10	11	12	
A	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

C6 is connected and ready.

Run Settings: Run with Limits, 0 events, 2 Min 30 Sec, 0 µL, Do not collect events outside P1.

Fluidics: Flow Rate 66 µL/min, Core Size 22 µm, Custom, Flow Rate 14 µL/min, Core Size 10 µm.

Threshold: 80,000 on FSC-H, None.

Statistics:

- Plot 1: A01 DMSO FITC + PI GATE [No Gating] - P1 88.7%
- Plot 7: A01 DMSO FITC + PI GATE (P1 in all) - Q1-UL 0.0%, Dead 12.6%, Live 84.5%, Apoptotic 2.5%
- Plot 9: A01 DMSO FITC + PI GATE (P1 in all) - Q5-UL 0.0%, Dead 12.7%, Live 84.4%

Select plot type to make a new plot.

Plot 1: A01 DMSO FITC + PI	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A
All	5,848	33	100.00%	100.00%	3,695,692.83	199,240.88	47.58%	80.00%	
P1	5,185	33	88.66%	88.66%	4,026,993.47	202,662.86	31.53%	42.41%	

Plot 7: A01 DMSO FITC + PI Gated on (P1 in all)	Count	Volume (µL)	% of This Plot	% of All	Mean FL1 Annexin V FITC-A	Mean FL3 Propidium Iodide-A	CV FL1 Ar
This Plot	5,185	33	100.00%	88.66%		261,139.65	72,673.25
Q1-UL	0	33	0.00%	0.00%		0.00	0.00
Dead	652	33	12.57%	11.15%	1,832,485.66	571,394.01	
Live	4,383	33	84.53%	74.95%	9,625.31	896.33	
Apoptotic	150	33	2.89%	2.56%	780,271.32	2,221.83	

Plot 9: A01 DMSO FITC + PI Gated on (P1 in all)	Count	Volume (µL)	% of This Plot	% of All	Mean FL1 Annexin V FITC-A	Mean FL3 Propidium Iodide-A	CV FL1 Ar
This Plot	5,185	33	100.00%	88.66%		261,139.65	72,673.25

Last Run: 0 Events, 0.00.0 Time, 0 Microliters, 0 Events / Sec, 0 Events / µL

Cumulative: 5,848 Events, 0.30.0 Time, 33 Microliters, 194 Events / Sec, 177 Events / µL

Delete Events: show warning

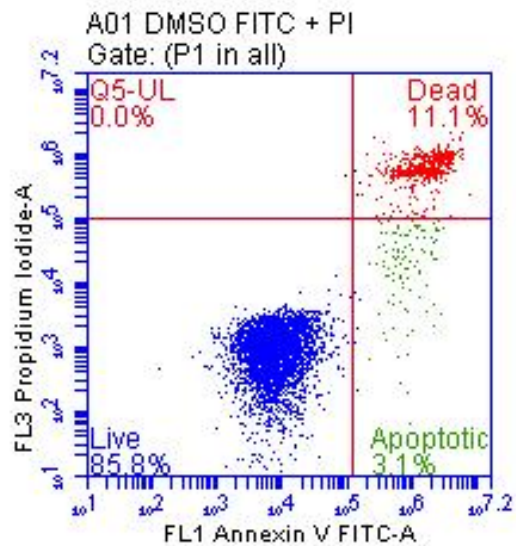
Data Capacity Used: <1% of 96,000,000 Events



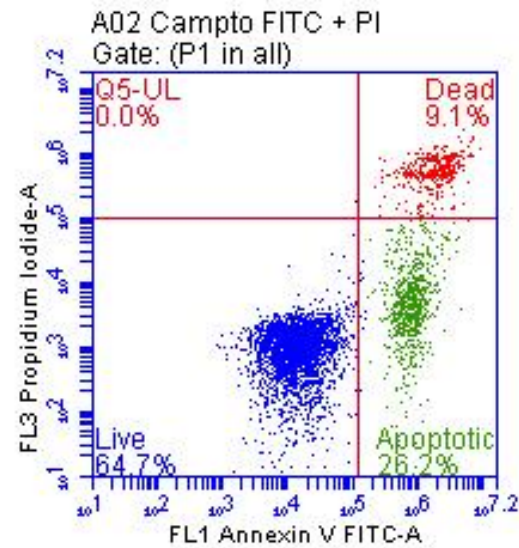
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Annexin V FITC Data

DMSO

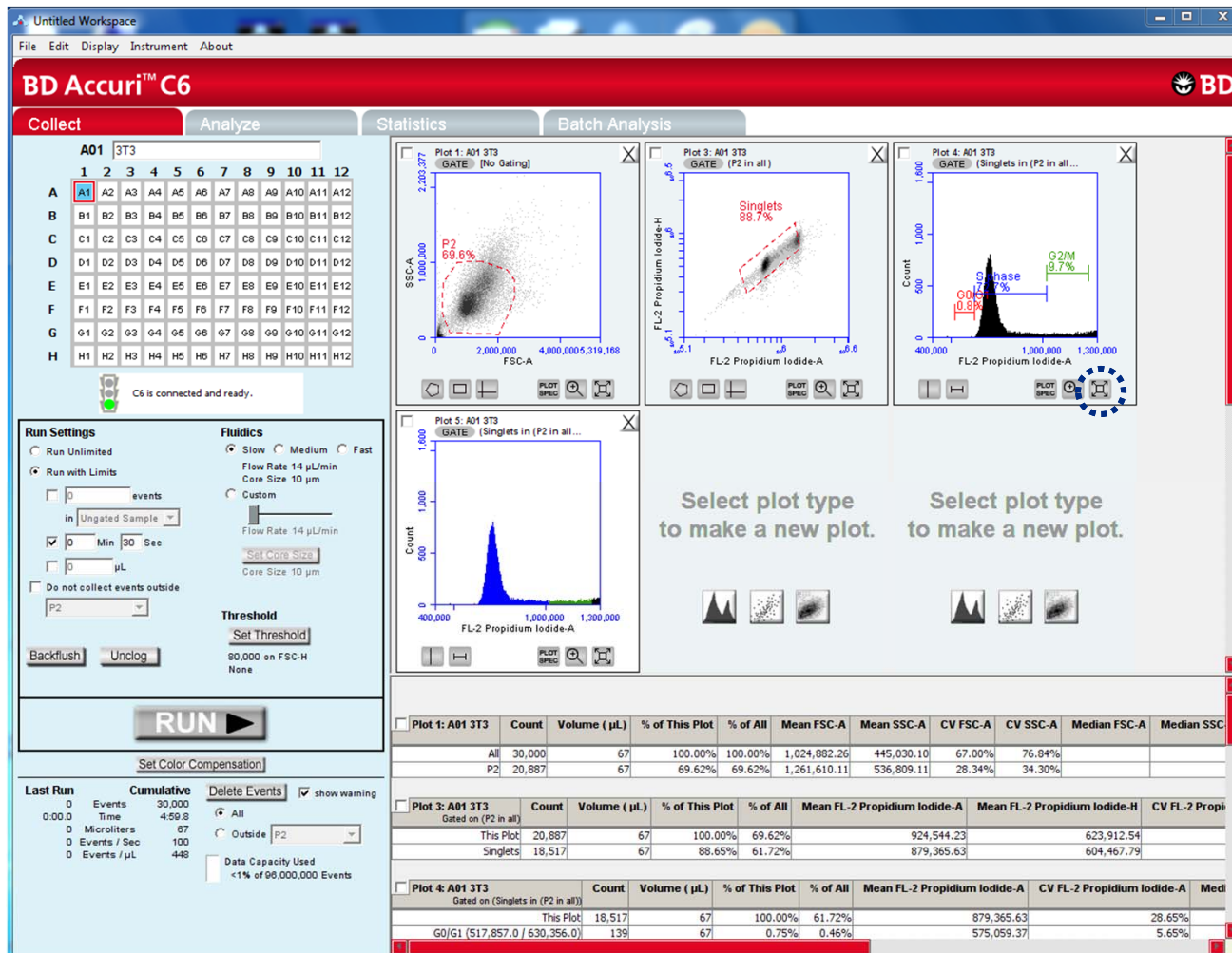


Camptothecin



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How to Further Optimize a Template: BD Accuri™ C6 Plus DNA Reagent Kit (Cat. No. 340202)



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How to Optimize a Template: Zoom Tool

BD Accuri™ C6

Collect Analyze Statistics Batch Analysis

A01 3T3

1 2 3 4 5 6 7 8 9 10 11 12

A A1 A2 A3 A4 A5 A6 A7 A8 A9 A10 A11 A12

B B1 B2 B3 B4 B5 B6 B7 B8 B9 B10 B11 B12

C C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 C11 C12

D D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12

E E1 E2 E3 E4 E5 E6 E7 E8 E9 E10 E11 E12

F F1 F2 F3 F4 F5 F6 F7 F8 F9 F10 F11 F12

G G1 G2 G3 G4 G5 G6 G7 G8 G9 G10 G11 G12

H H1 H2 H3 H4 H5 H6 H7 H8 H9 H10 H11 H12

C6 is connected and ready.

Run Settings

Run Unlimited
 Run with Limits
 0 events
 in Ungated Sample
 0 Min 30 Sec
 0 µL
 Do not collect events outside P2

Fluidics

Slow Medium Fast
 Flow Rate 14 µL/min
 Core Size 10 µm
 Custom
 Flow Rate 14 µL/min
 Set Core Size
 Core Size 10 µm

Threshold

Set Threshold
 80,000 on FSC-H
 None

Backflush Unclog

RUN

Set Color Compensation

Last Run

Cumulative
 0 Events 30,000
 0:00.0 Time 4:59.8
 0 Microliters 67
 0 Events / Sec 100
 0 Events / µL 448

Delete Events show warning
 All
 Outside P2

Data Capacity Used
 <1% of 96,000,000 Events

Plot 1: A01 3T3
 GATE: [No Gating]
 P2 74.3%

Plot 3: A01 3T3
 GATE: (P2 in all)

Plot 4: A01 3T3
 GATE: (Singlets in (P2 in all...))
 G0/G1 53.7%
 S phase 19.8%
 G2/M 26.8%

Plot 5: A01 3T3
 GATE: (Singlets in (P2 in all...))

Select plot type to make a new plot.

Select plot type to make a new plot.

Plot 4: A01 3T3 Gated on (Singlets in (P2 in all))	Count	Volume (µL)	% of This Plot	% of All	Mean FL-2 Propidium Iodide-A	CV FL-2 Propidium Iodide-A	Med
This Plot	21,381	67	100.00%	71.27%	957,860.97	31.52%	
G0/G1 (617,488.0 / 798,832.0)	11,479	67	53.69%	38.26%	714,508.97	4.47%	
S phase (791,858.0 / 1,280,093.0)	4,236	67	19.81%	14.12%	1,028,446.84	14.85%	
G2/M (1,274,548.0 / 1,573,035.0)	5,724	67	26.77%	19.08%	1,395,675.75	4.41%	

Plot 5: A01 3T3 Gated on (Singlets in (P2 in all))	Count	Volume (µL)	% of This Plot	% of All	Mean FL-2 Propidium Iodide-A	CV FL-2 Propidium Iodide-A	Med
This Plot	21,381	67	100.00%	71.27%	957,860.97	31.52%	




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How to Optimize a Template: Statistics Tab

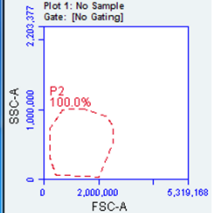
Untitled Workspace

File Edit Display Instrument About

BD Accuri™ C6 

Collect Analyze **Statistics** Batch Analysis

Plot 1: No Sample Gate: [No Gating]



Display Plot Preview

Plot 1 (FSC-A/SSC-A)
Plot 3 (FL2-A/FL2-H)
Plot 4 (FL2-A)
Plot 5 (FL2-A)

Statistics Column Selector
Add columns to your master statistics table by selecting a cell.

Plot 1 (FSC-A/SSC-A)	Count	Volume (µL)	Events / µL	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
P2	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Plot 3 (FL2-A/FL2-H)	Count	Volume (µL)	Events / µL	% of This Plot	% of All	Mean FL2-A	Mean FL2-H	CV FL2-A	CV FL2-H	Median FL2-A	Median FL2-H
This Plot	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Singlets	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Plot 4 (FL2-A)	Count	Volume (µL)	Events / µL	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A			
This Plot	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>			
G0/G1	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>			
S phase	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>			
G2/M	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>			
Plot 5 (FL2-A)	Count	Volume (µL)	Events / µL	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A			
This Plot	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>			

Sample Selector
Add rows to your master statistics table by selecting samples.

Preview	Add to Table	Sample Name
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	All Samples
<input type="checkbox"/>	<input checked="" type="checkbox"/>	H12 3T3

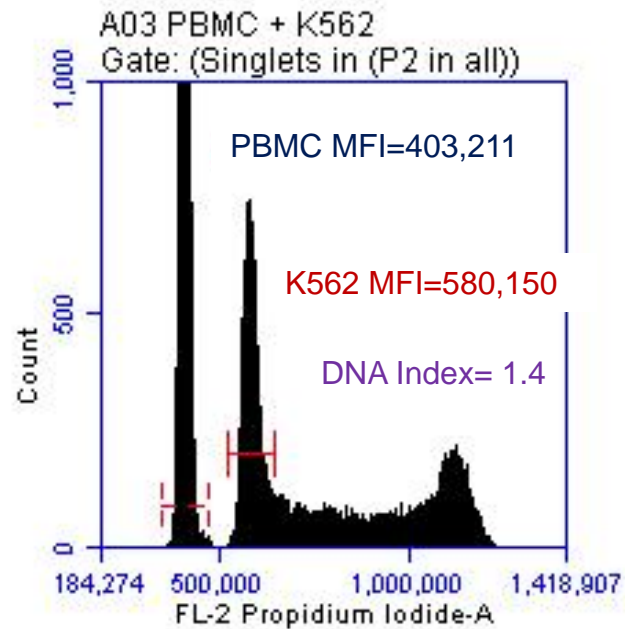
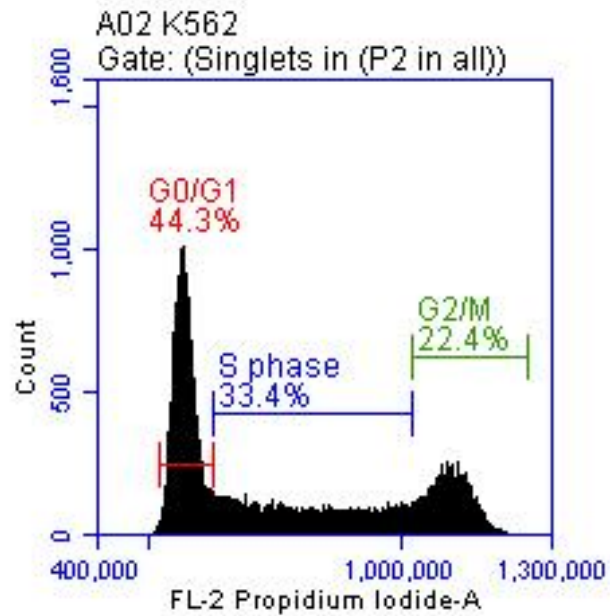
Master Statistics Table
Select cells from the Sample Selector and Statistics Column Selector to build your table.

	Plot 4 (FL2-A)		
	G0/G1	S phase	G2/M
% of This Plot	% of This Plot	% of This Plot	% of This Plot
H12 3T3	58.26%	18.16%	22.85%



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BD Cycletest Plus Data



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How to Optimize a Template for the BD CSampler™ Accessory: BD Pharmingen™ Active Caspase-3 PE (Cat. No. 550914)

The screenshot displays the BD CSampler software interface. On the left, a plate layout grid shows a 24-tube rack with well A1 selected. Below the grid are 'Run Settings' (Run Unlimited, Run with Limits) and 'Fluidics' (Slow, Medium, Fast) options. The main area shows two plots: 'Plot 1: A01 DMSO (GATE) [No Gating]' and 'Plot 2: A01 DMSO (GATE) (P1 in all)'. Plot 2 shows a histogram of 'PE active caspase-3-A' with a gate for 'Caspase 3-' (96.3%) and 'Caspase 3+' (3.7%). Below the plots are three prompts: 'Select plot type to make a new plot.' At the bottom, a statistics table provides data for both plots.

Plot 1: A01 DMSO	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	30,876	33	100.00%	100.00%	1,594,450.96	294,137.02	52.98%	73.83%		
P1	29,946	33	96.99%	96.99%	1,604,067.41	292,311.28	42.20%	51.34%		

Plot 2: A01 DMSO	Count	Volume (µL)	% of This Plot	% of All	Mean PE active caspase-3-A	CV PE active caspase-3-A	Median PE active caspase-3-A
Gated on (P1 in all)							
This Plot	29,946	33	100.00%	96.99%	3,863.78	709.46%	
Caspase 3- (1.0 / 7.8...	28,843	33	96.32%	93.42%	2,331.61	46.38%	
Caspase 3+ (7,818.0 / ...	1,103	33	3.68%	3.57%	43,929.43	311.32%	



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How to Optimize a Template: BD CSampler

BD CSampler™

Manual Collect | Auto Collect | Analyze | Statistics | Batch Analysis

Plate Type: 24 tube rack | Eject Plate

Plate Name: Click here to name plate

A01 DMSO

1	2	3	4	5	6	7	8	9	10	11	12	
A	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

C6 is connected and ready.

Run Settings

Run Unlimited | Run with Limits

0 events in Ungated Sample

0 Min 30 Sec

0 µL

Do not collect events outside P1

Backflush | Unclog | Wash | Agitate

Fluidics

Slow | Medium | Fast

Flow Rate 85 µL/min

Core Size 22 µm

Custom

Flow Rate 14 µL/min

Set Core Size

Core Size 10 µm

Threshold

Set Threshold

80,000 on FSC-H

None

ADD to A01

Set Color Compensation

Last Run

0	Events	30,876
0:0:0	Time	0:30:0
0	Microliters	33
0	Events / Sec	1,028
0	Events / µL	936

Data Capacity Used <1% of 24,000,000 Events

Plot 1: A01 DMSO GATE [No Gating]

P1 97.3%

Plot 2: A01 DMSO GATE [P1 in all]

Caspase 3- 96.2%

Caspase 3+ 3.8%

Plot 3: A01 DMSO GATE [No Gating]

Select plot type to make a new plot.

Plot 2: A01 DMSO Gated on (P1 in all)	Count	Volume (µL)	% of This Plot	% of All	Mean FL2 active caspase-3 PE-A	CV FL2 active caspase-3 PE-A	Median FL2
This Plot	30,054	33	100.00%	97.34%	3,879.54	705.24%	
Caspase 3 - (1.0 / 7.8...	28,903	33	96.17%	93.61%	2,332.27	46.53%	
Caspase 3+ (7,818.0 / ...)	1,151	33	3.83%	3.73%	42,733.31	313.49%	

Plot 3: A01 DMSO	Count	Volume (µL)	% of This Plot	% of All	Mean FL2 active caspase-3 PE-A	Mean SSC-A	CV FL2 active caspase-3 PE-A
All	30,876	33	100.00%	100.00%	4,317.20	294,137.02	1,074.78%



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How to Optimize a Template: BD CSampler

File Edit Display Instrument About

BD CSampler™

Manual Collect | **Auto Collect** | Analyze | Statistics | Batch Analysis

Plate Type: 24 tube rack Eject Plate

Plate Name: [Click here to name plate](#)

[Select All](#) [Deselect All](#)

Ctrl-click to view sample settings.

	1	2	3	4	5	6	7	8	9	10	11	12
A	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

C6 is connected and ready.

Run Limits

10000 events

in Ungated Sample

3 Min 0 Sec

0 µL

Do not collect events outside P1

Fluidics

Slow Medium Fast

Flow Rate: 14 µL/min

Core Size: 10 µm

Set Threshold

Delete events on (Minimum=10)

FSC-H less than 80000

less than 0

Wash Settings

None

Apply Settings Remove Settings

Agitate Plate

None every 1 Well(s) Run Horizontally

None every 1 Min Run Vertically

OPEN RUN DISPLAY

Well	Sample Name	Rename FL1	Rename FL2	Rename FL3	Rename FL4	Notes
A01	DMSO		FL2 active caspase-3 PE			
A02	camptothecin		FL2 active caspase-3 PE			
A03			FL2 active caspase-3 PE			
A04			FL2 active caspase-3 PE			
A05			FL2 active caspase-3 PE			
A06			FL2 active caspase-3 PE			
B01			FL2 active caspase-3 PE			
B02			FL2 active caspase-3 PE			
B03			FL2 active caspase-3 PE			
B04			FL2 active caspase-3 PE			
B05			FL2 active caspase-3 PE			
B06			FL2 active caspase-3 PE			
C01			FL2 active caspase-3 PE			
C02			FL2 active caspase-3 PE			
C03			FL2 active caspase-3 PE			
C04			FL2 active caspase-3 PE			
C05			FL2 active caspase-3 PE			
C06			FL2 active caspase-3 PE			
D01			FL2 active caspase-3 PE			
D02			FL2 active caspase-3 PE			
D03			FL2 active caspase-3 PE			
D04			FL2 active caspase-3 PE			
D05			FL2 active caspase-3 PE			
D06			FL2 active caspase-3 PE			



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How to Optimize a Template: BD CSampler

File Edit Display Instrument About

BD CSampler™

Manual Collect Auto Collect Analyze Statistics Batch Analysis

Plate Type: 24 tube rack Eject Plate
 Plate Name: Click here to name plate

Ctrl-click to view sample settings. [Select All](#) [Deselect All](#)

	1	2	3	4	5	6	7	8	9	10	11	12
A	✓	✓	✓	✓	✓	A6	A7	A8	A9	A10	A11	A12
B	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

C6 is connected and ready.

Run Limits
 10000 events
 Plate Full
 3 Min 0 Sec
 0 µL

Fluorescence
 Slow Medium Fast
 Flow Rate: 0.6 µL/min
 Cyt. Flow: 22 µL

Set Threshold
 Delete events on (Minimum=10)
 Do not collect events outside
 P1 FSC-H less than 80000
 less than 0

Wash Settings
 None Apply Settings Remove Settings

Agitate Plate
 None every 1 Well(s) Run Horizontally
 None every 1 Min Run Vertically

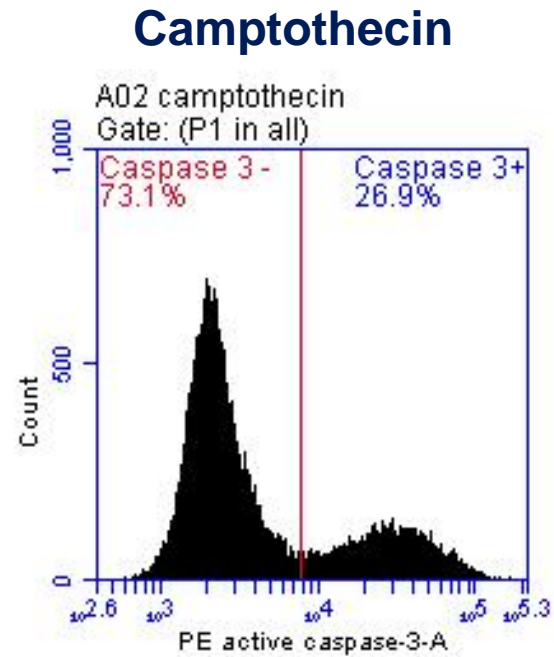
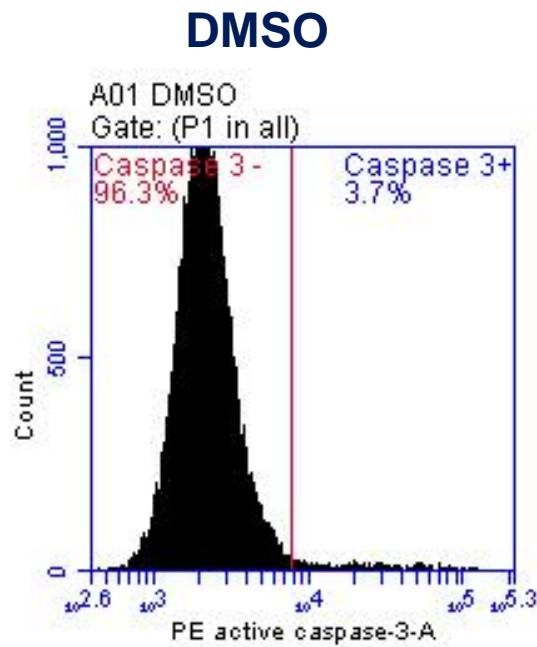
OPEN RUN DISPLAY

Well	Sample Name	Rename FL1	Rename FL2	Rename FL3	Rename FL4	Notes
A01	DMSO		FL2 active caspase-3 PE			
A02	camptothecin		FL2 active caspase-3 PE			
A03	compound 2		FL2 active caspase-3 PE			
A04	compound 3		FL2 active caspase-3 PE			
A05	compound 4		FL2 active caspase-3 PE			
A06			FL2 active caspase-3 PE			
B01			FL2 active caspase-3 PE			
B02			FL2 active caspase-3 PE			
B03			FL2 active caspase-3 PE			
B04			FL2 active caspase-3 PE			
B05			FL2 active caspase-3 PE			
B06			FL2 active caspase-3 PE			
C01			FL2 active caspase-3 PE			
C02			FL2 active caspase-3 PE			
C03			FL2 active caspase-3 PE			
C04			FL2 active caspase-3 PE			
C05			FL2 active caspase-3 PE			
C06			FL2 active caspase-3 PE			
D01			FL2 active caspase-3 PE			
D02			FL2 active caspase-3 PE			
D03			FL2 active caspase-3 PE			
D04			FL2 active caspase-3 PE			
D05			FL2 active caspase-3 PE			
D06			FL2 active caspase-3 PE			



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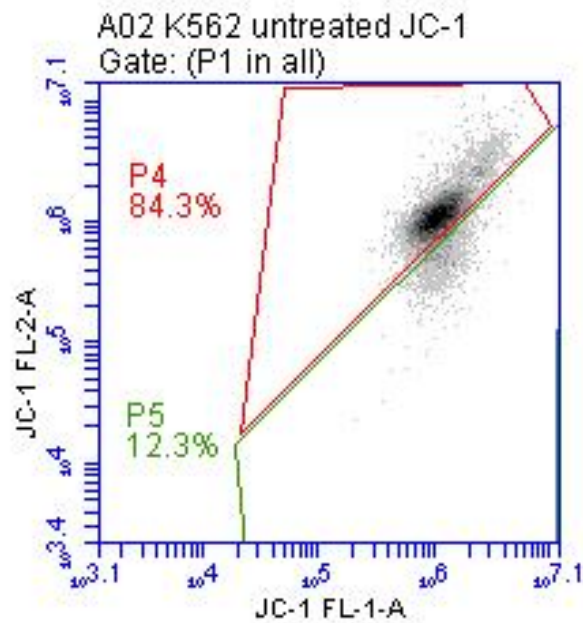
BD Pharmingen™ Active Caspase-3 PE Kit Data



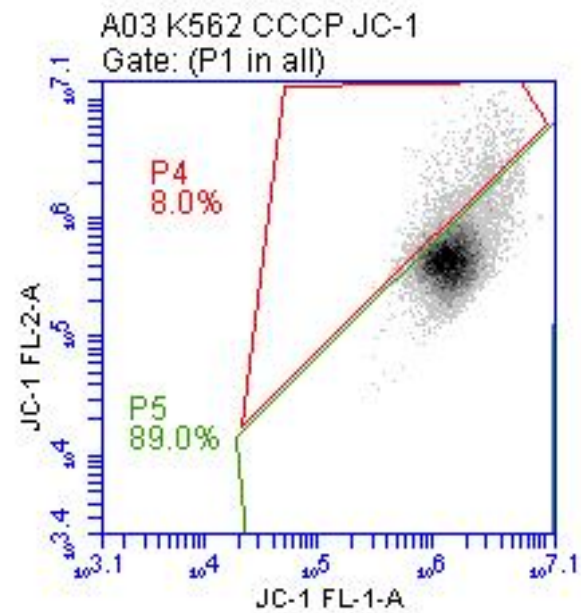
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Application-specific Templates: BD MitoScreen (Cat. No. 551302)

Untreated

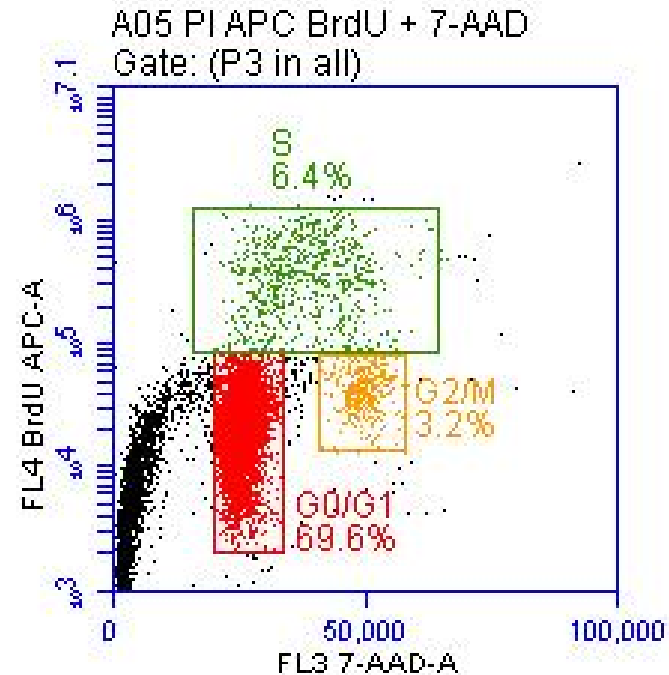
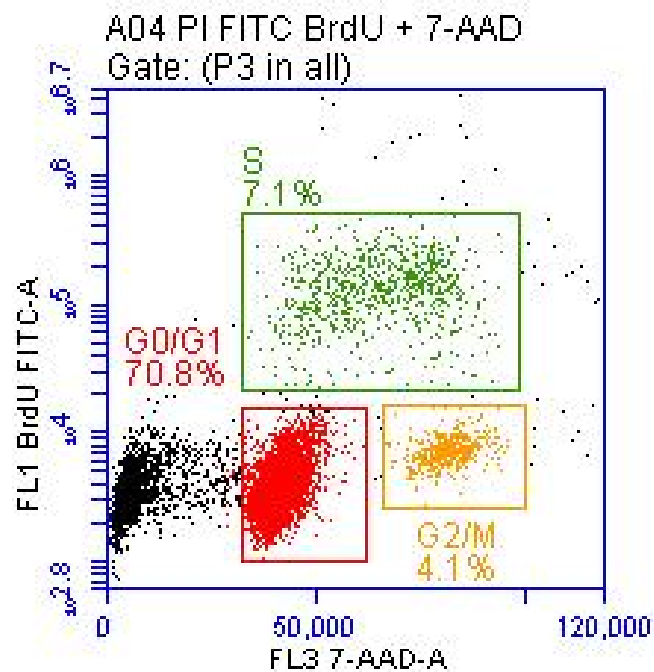


CCCP



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Application-specific Templates: BD Pharmingen™ FITC or APC BrdU Flow Kits (Cat. No. 559619 or 552598)



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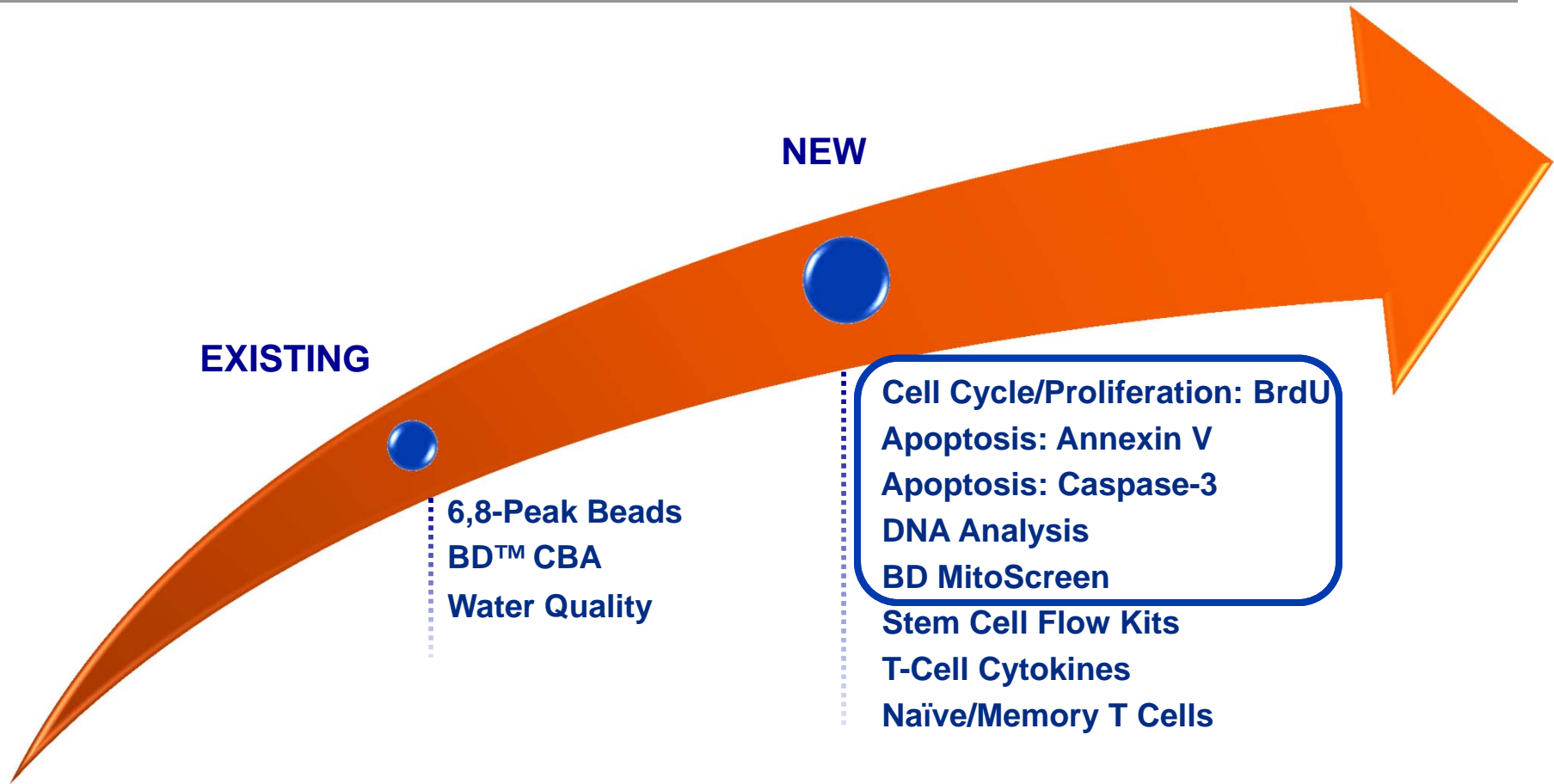
Outline

- Background: Apoptosis
- Introduction to the BD Accuri™ C6 Flow Cytometer
- BD Kits and Templates
- **BD Templates on the Web**
- How to Create Your Own Templates
- Coming Soon....



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BD Templates Available for Free on the Web



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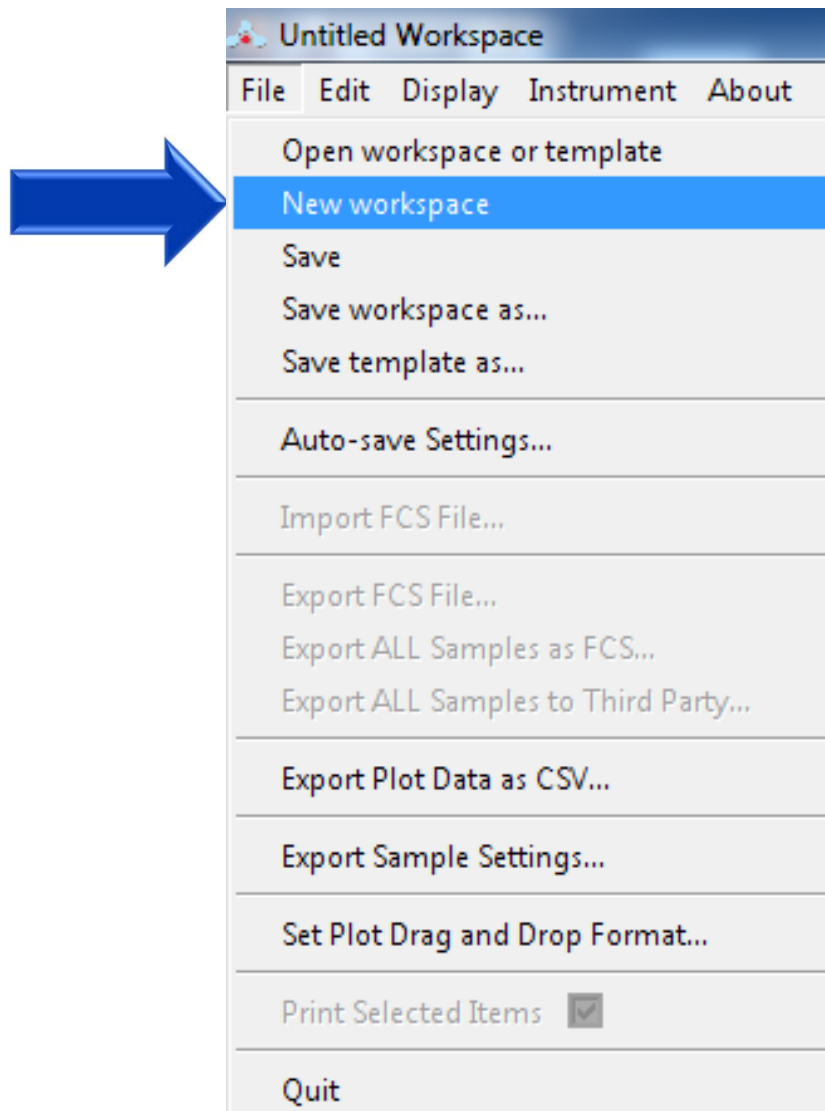
Outline

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How to Create Your Own Templates



1. Open a new workspace or existing workspace.



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How to Create Your Own Templates

Compensation Settings for Multitest 340571 4 color

Correct FL1 by subtracting a percentage of:

FL2: 5.00 % FL3: 0.00 % FL4: 0.00 %

Correct FL2 by subtracting a percentage of:

FL1: 7.70 % FL3: 0.00 % FL4: 0.00 %

Correct FL3 by subtracting a percentage of:

FL1: 0.00 % FL2: 22.00 % FL4: 0.00 %

Correct FL4 by subtracting a percentage of:

FL1: 0.00 % FL2: 0.20 % FL3: 2.00 %

Apply to:

Multitest... All samples

Reset all to 0.00% Preview Apply & Close Cancel & Close

2. Collect samples, optimize plots and run criteria to desired settings.
3. If desired, input compensation, parameter names, and threshold settings and click Apply to: All samples.

Rename Parameters

Rename FL1 to FL1 CD45RA FITC (FL1) Select...

Rename FL2 to FL2 CD45RO PE (FL2) Select...

Rename FL3 to FL3 CD3 PerCP (FL3) Select...

Rename FL4 to FL4 CD4 APC (FL4) Select...

Apply to:

Multitest 340571 4 color All samples

OK Cancel

Threshold Settings for Multitest 340571 4 color

PRIMARY THRESHOLD
Permanently eliminate events on
FSC-H less than 250000 (Range Minimum = 10)

OPTIONAL SECONDARY THRESHOLD
Permanently eliminate additional events on
- less than 0 (Range Minimum = 10)

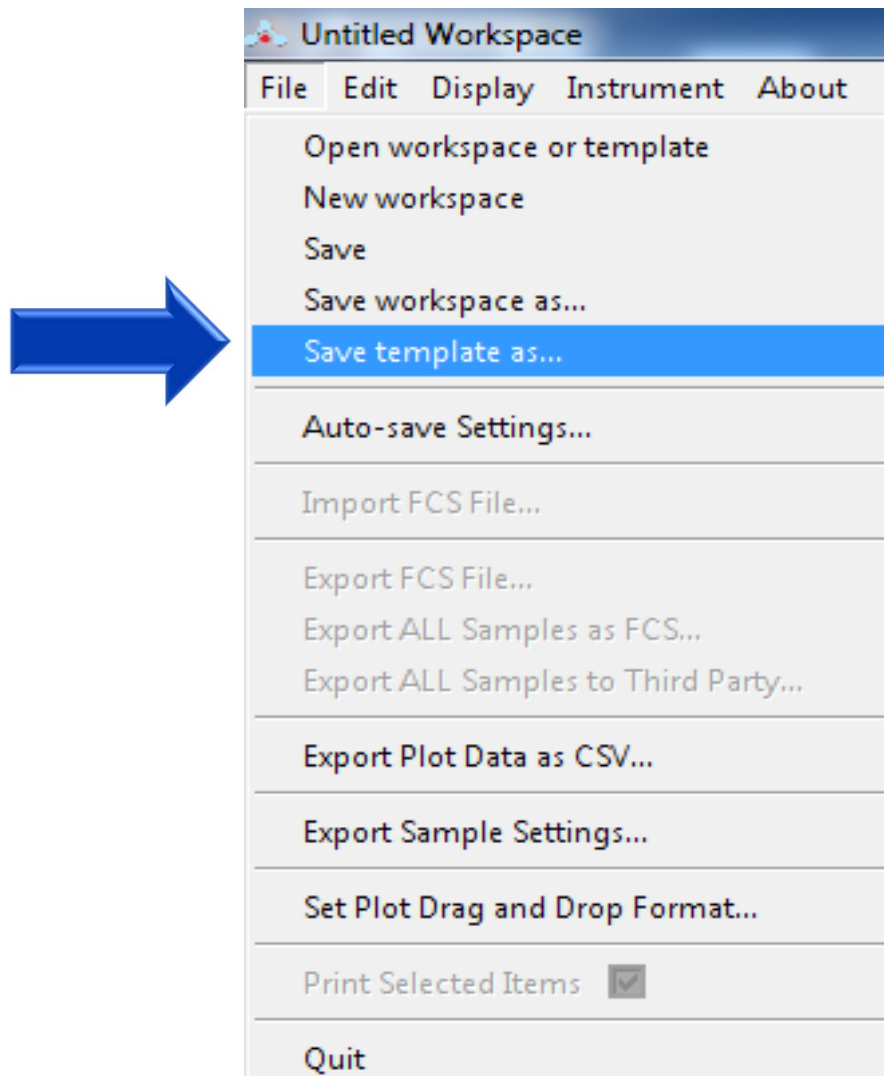
Apply to Only this sample All samples

Apply Close



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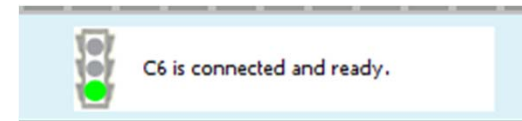
How to Create Your Own Templates



4. Select File > Save Template As.

5. Save the template with a new name.

Note: Creating templates works best at the cytometer workstation, when the BD Accuri C6 is powered on.



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Important Points to Remember

- Customize templates to fit your sample type and workflow.
- Verify all settings, including thresholds and compensation.
- When using the BD CSampler, copy desired settings to the Auto Collect Tab.
- For best results, create templates when the software is connected to the cytometer.



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Outline

- Background: Apoptosis
- Introduction to the BD Accuri™ C6 Flow Cytometer
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- **Coming Soon....**



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BD Templates Coming Soon to the Web.....



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Special Promotional Offer

For a limited time, take advantage of **big savings** and value-added packages for the BD Accuri™ C6 flow cytometer with our reagent kit discount.

Get a **40% discount on select reagent kits** now through June 2014.

New to BD Accuri?

Buy a BD Accuri C6 now and receive an immediate **10% discount** on the purchase price. You'll also be eligible to receive **40% off the list price of all BD Pharmingen™ reagents** you purchase for use on the BD Accuri C6 for the next 2 years.

Offer expires June 30, 2014.

Now you can simplify your workflow while dramatically reducing the cost of use of your personal flow cytometer.

www.bdbiosciences.com/go/templates

Summary

- The **BD Accuri™ C6** is making it even easier to apply the power of flow cytometry to your research with **free software templates** and **ready-to-go reagent kits** specific to your studies.
- Find out more about how the BD Accuri C6 puts the power of 4-color cell analysis within reach by visiting www.bdbiosciences.com/go/templates



Flow Cytometry Within Reach.™



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For More Information...

If you have further questions:

Contact Technical Support (US) at:

877-232-8995, Prompt 3, 2

or email: ResearchApplications@bd.com

Please visit our BD Accuri resources site at:

www.bdbiosciences.com/resources/accuri.

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