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

Presented by:
Stacey Roys, Scientist, BD Biosciences
Lora Tirri, BD Accuri Product Manager, BD Biosciences
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....
Outline

• **Background: Apoptosis and Cell Cycle**
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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
Apoptosis at a Glance

1. Phosphotidyl serine (PS) exposed
2. Mitochondrial potential decreases
3. Caspases activated
4. DNA fragmentation
## Summary of Apoptosis Assays

<table>
<thead>
<tr>
<th>Feature Measured</th>
<th>Assay</th>
<th>Key Benefits</th>
</tr>
</thead>
</table>
| Plasma Membrane Alterations      | Annexin V Binding Assay:             | • Detects early apoptosis  
• Quick and easy                                                                  |
|                                  | • Single Conjugates  
• Annexin V Kits                  |                                                                                                                                               |
| Mitochondrial Changes            | • BD™ MitoScreen                     | • Quick and easy                                                                                                                                  |
| Caspase Activation               | • Active Caspase-3 Flow Kit          | • Specific antibodies can detect activated vs uncleaved caspase-3  
• Can be multiplexed                                                              |
| DNA Fragmentation                | • APO-BrdU TUNEL Assay  
• APO-Direct TUNEL Assay          | • Works with adherent cells                                                                                                                                 |

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
- Decreases forward scatter

Untreated

Camptothecin treated
Apoptosis Method Decision Tree

**Cells**

**Flow**

**Unfixed**
- Loss of membrane symmetry: Annexin V*
- Mitochondrial membrane potential: JC1*
- Caspase inhibitors addition: VAD-FMK*

**Fixed**
- Cleaved Markers: Active Caspase 3*, PARP*
- DNA fragmentation: TUNEL assay/APO-BrdU kit*

* BD products available
Cell Cycle and Proliferation Analysis

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

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

The BD Accuri™ C6 Flow Cytometer System

An Affordable, Full-Featured, Easy-to-Use Flow Cytometer
Two lasers and six detectors
Intuitive Software

Sample Grid

Cytometer Status

Fluidics Controls

Run Criteria

Real-Time Updates

Histograms

Dot Plots

Density Plots

Analysis and Gating Tools

Plot Statistics
Outline

• Background: Immunophenotyping
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• **BD Kits and Templates**
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Arguably, as easy as cell analysis is going to get.
## BD Cell Biology Kits

<table>
<thead>
<tr>
<th>Application</th>
<th>Kit</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Apoptosis</strong></td>
<td>Annexin V FITC Apoptosis Detection Kit II</td>
<td>556570</td>
</tr>
<tr>
<td></td>
<td>Annexin V PE Apoptosis Detection Kit I</td>
<td>559763</td>
</tr>
<tr>
<td></td>
<td>MitoScreen (JC-1) Kit</td>
<td>551302</td>
</tr>
<tr>
<td></td>
<td>Caspase-3 PE Assay Kit</td>
<td>550914</td>
</tr>
<tr>
<td><strong>Cell Cycle and DNA</strong></td>
<td>DNA Reagent Kit</td>
<td>340242</td>
</tr>
<tr>
<td></td>
<td>FITC BrdU Flow Kit</td>
<td>559619</td>
</tr>
<tr>
<td></td>
<td>APC BrdU Flow Kit</td>
<td>552598</td>
</tr>
</tbody>
</table>
A BD Accuri™ template is a pre-defined workspace that includes gates, labels, run criteria, and compensation settings for a specific assay.
Pre-Defined Gating and Run Criteria for Ease of Use
### Compensation

![BD Accuri C6 Software Interface](image)

#### Compensation Settings for A11:

Correct FL1 by subtracting a percentage of:

- FL1: 0.00 %
- FL2: 0.00 %
- FL3: 0.00 %
- FL4: 0.00 %

Apply to:

- FL1
- FL2
- FL3
- FL4

Select plot type to make a new plot.

#### Data Table:

<table>
<thead>
<tr>
<th>Plot Type</th>
<th>Events</th>
<th>Volume (μL)</th>
<th>% of This Plot</th>
<th>% of All</th>
<th>Mean FSC.M</th>
<th>Mean SSC.M</th>
<th>CV FSC.M</th>
<th>CV SSC.M</th>
<th>Median FSC.M</th>
<th>Median SSC.M</th>
</tr>
</thead>
<tbody>
<tr>
<td>A11</td>
<td>0</td>
<td>0</td>
<td>100.00 %</td>
<td>100.00 %</td>
<td>0.00</td>
<td>0.00</td>
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<td>0.00</td>
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<tr>
<td>A12</td>
<td>0</td>
<td>0</td>
<td>100.00 %</td>
<td>100.00 %</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>All</td>
<td>0</td>
<td>0</td>
<td>100.00 %</td>
<td>100.00 %</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

[BD Logo]

Helping all people live healthy lives
# Where to Find the Templates

[www.bdbiosciences.com/go/templates](http://www.bdbiosciences.com/go/templates)

<table>
<thead>
<tr>
<th>Category</th>
<th>Product Information Sheet</th>
<th>Brand</th>
<th>Kit</th>
<th>Cat. No.</th>
<th>Template</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Biology</td>
<td>BD Apoptosis Kits and Templates</td>
<td>BD Pharmingen™</td>
<td>Annexin V FITC Apoptosis Detection Kit II</td>
<td>556570</td>
<td>Download</td>
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<tr>
<td></td>
<td></td>
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<td>Annexin V PE Apoptosis Detection Kit I</td>
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<td></td>
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<td>Caspase-3 PE Assay Kit</td>
<td>550914</td>
<td>Download</td>
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<tr>
<td>BD Cell Cycle and DNA Kits and Templates</td>
<td>BD Cycletest™ Plus</td>
<td>BD Cycletest™ Plus</td>
<td>DNA Reagent Kit</td>
<td>340242</td>
<td>Download</td>
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<td>BD Cycletest™ Plus</td>
<td>FITC BrdU Flow Kit</td>
<td>559619</td>
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<td>BD Cycletest™ Plus</td>
<td>APC BrdU Flow Kit</td>
<td>552598</td>
<td>Download</td>
</tr>
<tr>
<td>Immunology</td>
<td>BD Naïve/Memory T-Cell Kits and Templates</td>
<td>BD Multitest™</td>
<td>CD45RA/CD45RO/CD3/CD4</td>
<td>340571</td>
<td>Download</td>
</tr>
<tr>
<td>BD Naïve/Memory T-Cell Kits and Templates</td>
<td>BD Multitest™</td>
<td>BD Multitest™</td>
<td>CD45RA/CD62L/CD3/CD4</td>
<td>340977</td>
<td>Download</td>
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<tr>
<td>BD Naïve/Memory T-Cell Kits and Templates</td>
<td>BD Multitest™</td>
<td>BD Multitest™</td>
<td>Human Naïve/Memory T Cell Panel</td>
<td>561438</td>
<td>Download</td>
</tr>
<tr>
<td>BD Intracellular T-Cell Kits and templates</td>
<td>BD Multitest™</td>
<td>BD Pharmingen™</td>
<td>Th1/Th2/Th17 Phenotyping Kit</td>
<td>560751</td>
<td>Download</td>
</tr>
<tr>
<td>BD Intracellular T-Cell Kits and templates</td>
<td>BD FastImmune™</td>
<td>BD FastImmune™</td>
<td>IFN-γ/CD69/CD8/CD3 Kit</td>
<td>346048</td>
<td>Download</td>
</tr>
<tr>
<td>BD Intracellular T-Cell Kits and templates</td>
<td>BD FastImmune™</td>
<td>BD FastImmune™</td>
<td>IFN-γ/IL4 Kit</td>
<td>340456</td>
<td>Download</td>
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</table>
How to Open a Template
How to Open a Template
How to Use a Template: Example Using the Annexin V Apoptosis Detection Kit (Cat. No. 556570)

Reagents:
Annexin V FITC
Propidium Iodide
How to Use a Template: Verify Settings, Collect, Adjust as Necessary
Ready to Acquire Data
Annexin V FITC Data

**DMSO**

A01 DMSO FITC + PI
Gate: (P1 in all)

**Camptothecin**

A02 Campto FITC + PI
Gate: (P1 in all)
How to Further Optimize a Template:
BD Cycletest™ Plus DNA Reagent Kit (Cat. No. 340202)
How to Optimize a Template: Zoom Tool
How to Optimize a Template: Statistics Tab
**BD Cycletest Plus Data**

**A02 K562**
- **Gate:** (Singlets in (P2 in all))
- **G0/G1:** 44.3%
- **S phase:** 33.4%
- **G2/M:** 22.4%

**A03 PBMC + K562**
- **Gate:** (Singlets in (P2 in all))
- **PBMC MFI=403,211**
- **K562 MFI=580,150**
- **DNA Index= 1.4**
How to Optimize a Template for the BD CSampler™ Accessory: BD Pharmingen™ Active Caspase-3 PE (Cat. No. 550914)
How to Optimize a Template: BD CSampler
How to Optimize a Template: BD CSampler
How to Optimize a Template: BD CSampler
BD Pharmingen™ Active Caspase-3 PE Kit Data

DMSO

A01 DMSO Gate: (P1 in all)

Caspase 3- 96.3%
Caspase 3+ 3.7%

Camptothecin

A02 camptothecin Gate: (P1 in all)

Caspase 3- 73.1%
Caspase 3+ 26.9%
Application-specific Templates:
BD MitoScreen (Cat. No. 551302)

Untreated

A02 K562 untreated JC-1
Gate: (P1 in all)

P4 84.3%
P5 12.3%

CCCP

A03 K562 CCCP JC-1
Gate: (P1 in all)

P4 8.0%
P5 89.0%
Application-specific Templates: BD Pharmingen™
FITC or APC BrdU Flow Kits (Cat. No. 559619 or 552598)
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BD Templates Available for Free on the Web

EXISTING

6,8-Peak Beads
BD™ CBA
Water Quality

NEW

Cell Cycle/Proliferation: BrdU
Apoptosis: Annexin V
Apoptosis: Caspase-3
DNA Analysis
BD MitoScreen
Stem Cell Flow Kits
T-Cell Cytokines
Naïve/Memory T Cells
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How to Create Your Own Templates

1. Open a new workspace or existing workspace.
How to Create Your Own Templates

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

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- BD CBA
- Water Quality

NEW

- Cell Cycle/Proliferation: BrdU
- Apoptosis: Annexin V
- Apoptosis: Caspase-3
- DNA Analysis
- BD MitoScreen
- Stem Cell Flow Kits
- T-Cell Cytokines
- Naïve/Memory T Cells

COMING SOON

- Tregs
- Mouse Lymphocytes
- Rat Lymphocytes
- Mouse Tregs
- Mouse T-Cell Cytokines
- Counting and Viability of Bacteria
- Mesenchymal Stem Cell Kit
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.

wwwbdbiosciences.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](http://www.bdbiosciences.com/go/templates)
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.