Apoptosis Detection Using the BD Accuri™ C6 Flow Cytometer

Stacey Roys, Marketing Applications Specialist, BD Biosciences

Cyndy Lane, Senior Product Manager, BD Biosciences
Outline

• What is apoptosis?
• Methods of apoptosis detection
• Detection of apoptosis by flow cytometry
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
Hallmarks of Apoptosis

- Plasma membrane alterations
- Mitochondrial changes
- Activation of caspases
- DNA fragmentation
## Summary of Apoptosis Assays

<table>
<thead>
<tr>
<th>Feature Measured</th>
<th>Assay</th>
<th>Key Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Membrane Alterations</td>
<td>Annexin V Binding Assay:</td>
<td>• Detects early apoptosis</td>
</tr>
<tr>
<td></td>
<td>• Single Conjugates</td>
<td>• Quick and easy</td>
</tr>
<tr>
<td></td>
<td>• Annexin V Kits</td>
<td></td>
</tr>
<tr>
<td>Mitochondrial Changes</td>
<td>• BD MitoScreen</td>
<td>• Fast and easy</td>
</tr>
<tr>
<td>Caspase Activation</td>
<td>• Active Caspase-3 Flow Kit</td>
<td>• Specific antibodies can detect activated versus uncleaved caspase-3</td>
</tr>
<tr>
<td>DNA Fragmentation</td>
<td>• APO-BrdU TUNEL Assay</td>
<td>• Can be multiplexed</td>
</tr>
<tr>
<td></td>
<td>• APO-Direct TUNEL Assay</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Works with adherent cells</td>
</tr>
</tbody>
</table>
The BD Accuri™ C6 Flow Cytometer System

An affordable, full-featured, easy-to-use flow cytometer
Two lasers and six detectors
Optics

Compact optical system design reduces cost and eliminates alignment issues

488-nm solid state, 21-mW laser (Melles Griot/JDS Uniphase)

640-nm diode, 14.7-mW laser (custom manufacture)

PMTs for fluorescence detection

Diodes for scatter detection

User changeable optical filters

- 510/15 nm
- 540/20 nm
- 565/20 nm
- 610/20 nm
- 780/60 nm
Fluidics

- Microprocessor-controlled peristaltic pumps enable direct volume measurement
- Many types of sample tubes may be used
  - BD Falcon™ fluorescence activated cell sorting tubes
  - Microcentrifuge tubes
  - Ninety-six-well plates with the BD CSampler™ accessory
- Open system conducive for kinetic studies
Detection: Wide Dynamic Range
Intuitive Software

Sample Grid
Cytometer Status
Fluidics Controls
Run Criteria
Real-Time Updates

Histograms
Dot Plots
Density Plots
Analysis and Gating Tools
Plot Statistics
Why Choose the BD Accuri C6 for Apoptosis Measurements?

- Small footprint: fits on a benchtop
- Easy to use
- Locked-down alignment, no voltage or gain adjustments
- Minimal setup and QC
- Affordable
- Intuitive software
- Most apoptosis assays use fewer than four colors
Apoptosis: Scatter Properties

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

Formation of apoptotic vesicles
- Increases side scatter

Reduced refractive index of apoptotic cells
- Decreases forward scatter
Annexin V is a surface marker and detects early membrane changes associated with apoptosis.

**Pros:** Rapid confirmation of apoptosis
Uses live, unfixed cells

**Applications**
- Flow cytometry (cells in suspension)
- Fluorescence microscopy (adherent cells)
Jurkat T cells were treated with camptothecin for 4 hours and stained with Annexin V FITC + propidium iodide.
Detection of Apoptosis with JC-1

- Changes in mitochondrial potential are another early marker for apoptosis.
- Lipophilic cationic fluorochromes such as JC-1 penetrate cells and form aggregates.
- Monomers and aggregates of JC-1 have different emission spectra.
  - In healthy cells, JC-1 accumulates in the mitochondria and forms aggregates.
  - In apoptotic cells, JC-1 does not accumulate in the mitochondria and remains in the cytoplasm as monomers.
- Changes in membrane potential can be determined by comparing the ratio of fluorescence between the FL1 and FL2 channels.
K562 cells were treated with the compound CCCP (10 µM) for 10 minutes and stained with JC-1.
Caspase-3

- Caspases are proteases that are activated upon cleavage at aspartate residues at the earliest stages of apoptosis.
- Several caspases are important for apoptosis, including caspases-3, 8, and 9.
- Methods to measure caspase cleavage include fluorogenic substrates and detection with antibodies specific to the cleaved (activated) forms of caspases.
Active Caspase-3

Jurkat T cells were treated with either DMSO or camptothecin (6 µM) for 4 hours, then fixed, permeabilized and stained for active caspase-3.
APO-Direct Assay

DNA fragmentation is one of the last steps in apoptosis.

Fragmented DNA can be detected by the end labeling or TUNEL method.

- Enzymatic assay catalyzed by terminal deoxynucleotidyltransferase (TdT)
Detection of DNA Fragmentation During Apoptosis by “End Labeling” or “TUNEL” Using the BD APO-Direct Kit
# Summary of Apoptosis Detection Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annexin V</td>
<td>• Works well on live cells</td>
<td>• Difficult to multiplex</td>
</tr>
<tr>
<td></td>
<td>• Difficult to multiplex</td>
<td>• Requires calcium</td>
</tr>
<tr>
<td>BD MitoScreen (JC-1)</td>
<td>• Can be performed on live cells</td>
<td>• Difficult to multiplex</td>
</tr>
<tr>
<td></td>
<td>• Ratiometric data</td>
<td></td>
</tr>
<tr>
<td>Caspase-3</td>
<td>• Can be multiplexed with other markers</td>
<td>• Requires fixation and permeabilization of cells</td>
</tr>
<tr>
<td>BD APO-Direct</td>
<td>• Useful for detection of late apoptosis</td>
<td>• Difficult to multiplex</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Requires fixation and permeabilization of cells</td>
</tr>
</tbody>
</table>
Tips/Tricks

- Keep your system clean.
- Perform *daily* QC with 6- or 8-peak beads.
- Make use of well established positive controls (camptothecin, staurosporine, etc).
- Use light scatter to your advantage.
- Use the Zoom tool to quickly locate populations.
- Start with suggested compensation values.
- Create software templates for common applications.
## Suggested Compensation Values

<table>
<thead>
<tr>
<th></th>
<th>FITC</th>
<th>PE</th>
<th>PerCP</th>
<th>PerCP-Cy™5.5</th>
<th>PE-Cy™7</th>
<th>APC</th>
</tr>
</thead>
<tbody>
<tr>
<td>FL1 (530 BP)</td>
<td>-</td>
<td>3.50</td>
<td>0.00</td>
<td>0.00</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>FL2 (585 BP)</td>
<td>7.00</td>
<td>-</td>
<td>0.00</td>
<td>0.00</td>
<td>3.50</td>
<td>0.00</td>
</tr>
<tr>
<td>FL3 (670 LP)</td>
<td>1.00</td>
<td>14.50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.20</td>
</tr>
<tr>
<td>FL4 (675 BP)</td>
<td>0.00</td>
<td>0.00</td>
<td>3.00</td>
<td>12.00</td>
<td>0.00</td>
<td>-</td>
</tr>
</tbody>
</table>
Pre-optimized Detectors: Adjust View with Zoom
Conclusions

- There are multiple methods currently available to measure apoptosis.
- Apoptosis detection by flow cytometry can be done easily, using two colors.
- The BD Accuri C6 is a perfect analysis tool for new and experienced users.
Acknowledgments:

Maria Dinkelmann
Mette Ejrnaes
Monisha Sundararajan
Koen Verbrugghe