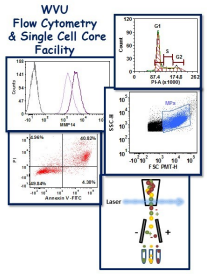
WVU FLOW CYTOMETRY & SINGLE CELL CORE FACILITY



Newsletter Volume 6, issue 3 January 2020

Apoptosis Assays

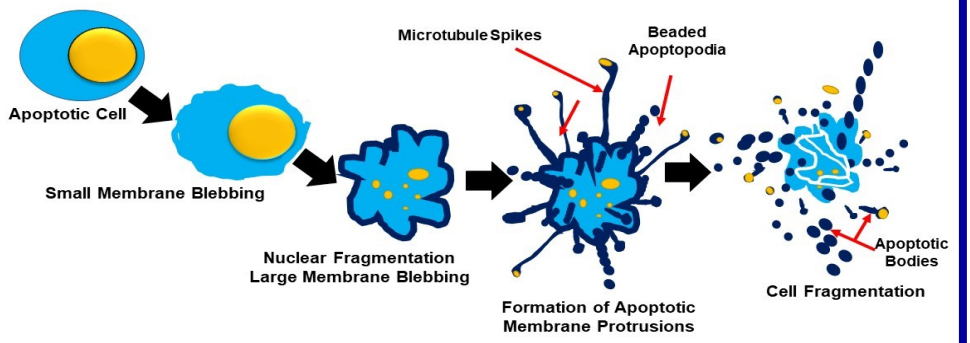


Figure 1. Changes to a cell as it undergoes apoptosis.

Cell death can occur by a form of programed cell death known as apoptosis, often referred to as Type 1 Cell Death. It is a highly regulated form of cell death used by multicellular organisms for normal growth and development as well as a way to remove old and unhealthy cells.

Flow cytometry is routinely used to detect apoptotic cells using a variety of cytometric assays. Some assays detect changes to a cell's membrane while other detect enzymes that become activated during the apoptotic process. In this article, the apoptotic process along with some common flow cytometry based apoptotic assays will be reviewed.

Cell Death

A cell's death is thought to occur by one of 4 processes, including apoptosis. Some cells die by a non-physiological process known as necrosis. Death by necrosis usually occurs as a result of an infection or injury. Another process by which a cell can die is pyroptosis, a lytic regulated cell death also known as inflammatory programmed cell death. Death by pyroptosis usually occurs during an infection with intracellular pathogens or as the result of pathological stimuli such as stroke or cancer. Autophagy is a form of programmed cell death often referred to as a Type 2 Cell Death. It is a highly regulated mechanism by which cells get rid of unneeded or non-functional cellular components.

Apoptosis

Figure 1 describes the changes a cell undergoes during apoptosis. Once a cell has been triggered to start the apoptosis process, the first noticeable changes occur at the cell membrane level. Initially, small membrane blebbing is observed with phosphatidylserine expression on the surface of the cell. In a healthy cell, phosphatidylserine is normally only found on the interior surface of the plasma membrane. The small membrane blebbing eventually start to form large membrane blebs. At the same time, the nucleus starts to fragment. The fragmentation of nuclear DNA results in multiples of 200 bp fragments which appears on agarose gel electrophoresis as a DNA laddering effect. As the cell proceeds

Inside this Issue

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Hours of operation:
9:30 am to 5:00 pm, M-F

After hours access is available for experienced users with prior approval from Dr. Kathy Brundage

Contact Dr. Brundage at:
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further down the apoptotic pathway, it starts to form apoptotic membrane protrusions including microtubule spikes and beaded apoptopedias. These beaded apoptopedias start as elongated protrusions that segment and eventually appear beaded. In the final stage of apoptosis, the cell completely fragments with the beaded apoptopedias breaking off. In many cases, these pieces of membrane, called apoptotic bodies, will contain pieces of the fragmented DNA produced earlier in the process.

Measuring Apoptosis

There are many assays available for detecting apoptotic cells using flow cytometry. The most commonly performed assay is the annexin V binding assay. This assay can detect cells that have just started down the apoptotic pathway when phosphatidylserines are exposed on the outside of the cell's plasma membrane. Annexin V is not an antibody. It is a protein made by bacteria that is fluorescently labeled and binds specifically to phosphatidylserine. Annexin V assays must be carried out in a buffer containing calcium because it is required for annexin V binding, unlike when using antibodies to stain cells. In addition to staining with annexin V, it is common practice to stain the cells with propidium iodide (PI) at the same time. The combination of annexin V and PI allows the researcher to determine how far down the apoptotic pathway the cells have progressed. Figure 2 is an example of an annexin V binding assay coupled with PI staining.

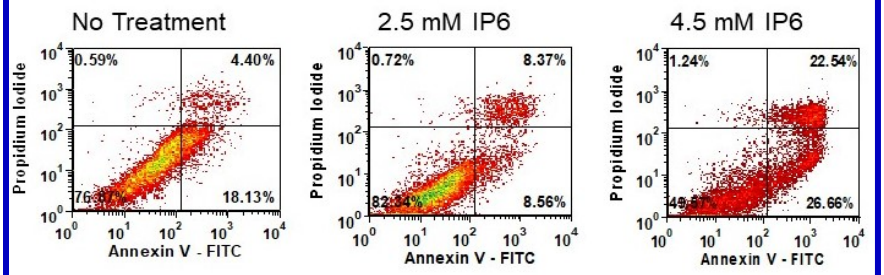


Figure 2. Example of an annexin V assay for apoptosis. Cells were exposed to 2 different dose of IP6 for 2 hours. Cells were harvested and labeled with annexin V-FITC and propidium iodide. Upper Left Quadrant – necrotic cells; Upper Right Quadrant - Late apoptosis/ necrosis; Lower Left Quadrant – Live cells; Lower Right Quadrant – Early apoptosis. Data from Dr. S. Kandzari's laboratory.

Other apoptotic assays can identify cells later in the apoptotic process. For example, there are assays that look for caspase enzyme activity. Typically in these assays a caspase sensitive substrate that fluoresces when cleaved is used. The most common caspase activity assays detect caspase 3 and 7 activity. Caspase 3 is a key component of the apoptotic pathway. It amplifies initial caspase signals such as caspase 8 and is the point of no return for the cell. In addition, caspase 3 cleaves poly(ADP-ribose) polymerase, DNA-dependent protein kinase, protein kinase Cδ and actin. There are also caspase activity assays that measure Caspase 8 and Caspase 9.

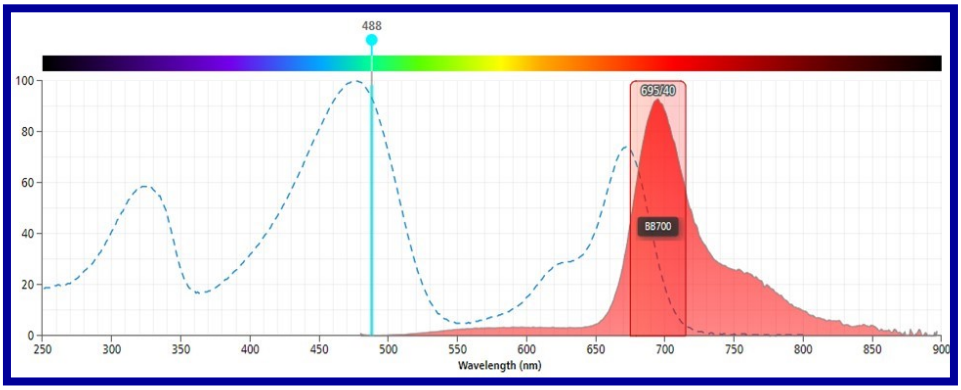
In addition to detecting caspase activity, there are kits available to measure caspase binding. These assays can detect caspase 1, 2, 3/7, 6, 8, 9, 10 and 13 binding using fluorescent probes. In these assays, binding of the fluorescent probes by caspase prevents the caspase from cleaving the probe and results in the probe being retained in the cell.

Fragmentation of the cell's DNA, which occurs late in the apoptotic process can be detected by flow cytometry. Usually this is done using a TUNEL assay. The TUNEL assay uses an enzyme terminal deoxynucleotidyl transferase to catalyze the addition of dUTP nucleotides to the free 3'-hydroxyl terminal end of double stranded fragmented DNA. Cell cycle analysis can also be used to detect cells undergoing apoptosis. In this assay, a peak of cells that appear below the Go/G1 peak is usually a good indication that some of the cells are apoptotic. If a sub-Go/G1 peak is detected in the cell cycle analysis, a second assay should be performed to confirm that the cells are apoptotic.

Finally, mitochondrial changes are another hallmark of apoptosis. Dyes such as JC-1 which fluoresces green when membrane potential is low and fluoresces red when membrane potential is high are often used or Mitotracker dyes which accumulates in mitochondria based on the mitochondrial membrane potential. There also are commercial kits that measure mitochondrial membrane potential using proprietary cationic probes.

A quick Pubmed search locates over 29,000 articles, published in 2019 alone, referencing apoptosis in a wide range of diseases and their treatment including breast cancer, myocardial infarction, and stroke. If you are interested in measuring apoptosis via flow cytometry and have any questions please make an appointment with me to discuss your project and needs.

Meet the Fluorochrome: BD Horizon Brilliant Blue 700 aka BB700



Excitation max

485 nm (Blue laser)

Emission max

693 nm (same detector as the one used for PerCp-Cy5.5)

Type of Fluorochrome: Polymer based tandem dye

Characteristics: Brighter than PerCp-Cy5.5

Minimal excitation by the Violet laser so minimal spillover into the Violet detectors which means less compensation issues

Upcoming Holidays & Events

January 20, 2020	MLK Holiday	Facility Closed
January 31— February 7, 2020	Kathy out of the Lab	Facility Open for Experienced Users Only No sorting
February 28— March 4, 2020	Kathy out of the Lab	

New User Guide

Hands-on training for LSRFortessa, C1 Single Cell Auto Prep System, NanoSight NS300 and Zetasizer Nano Z is **mandatory** for all new users and must be scheduled by consultation with the facility director.

Training will initiate with user's first experiment. Due to the complexity of the instruments and software, facility staff will fully assist with the acquisition of the first dataset and will continue with additional assistance on a "needs" basis until users are comfortable operating the instrument on their own. Sorting on the FACSAria is by facility staff only.

Note to users:

Please remember to acknowledge the support of the HSC Research Office and NIH grants that support the WVU Flow Cytometry & Single Cell Core in all your publications. The grant numbers are listed below:

TME CoBRE grant: P20GM121322

WVCTS grant: GM104942 important if you used the Miltenyi AutoMACS pro (installed 6/29/18)

WV InBRE grant: GM103434

Fortessa S10 grant: OD016165

NanoSight NS 300 use Stroke CoBRE grant GM109098 and WVCTS grant GM104942

ZetaSizer NanoZ use Stroke CoBRE grant GM109098 and WVCTS grant GM104942

Flow Cytometers in the Facility

FACSAria III Cell Sorter

Operator: Facility Staff

Lasers:

- 488 nm solid state
- 561 nm solid state
- 633 nm solid state
- 407 nm solid state

Detection Parameters:

- Forward Scatter
- Side Scatter
- Simultaneous detection of 13 fluorochromes

Applications:

- Cell Sorting (Aseptic)
- Single Cell Sorting
- Cell Phenotyping
- Cell Viability
- Cell Cycle Analysis

LSR Fortessa

Operator: User of Facility Staff

Lasers:

- 405 nm solid state
- 488 nm solid state
- 561 nm solid state
- 628 nm solid state

Detection Parameters:

- Forward Scatter
- Side Scatter
- Simultaneous detection of 17 fluorochromes

Applications:

- Cell phenotyping
- Cell Viability
- Cell Cycle analysis
- Apoptosis Assays

Other Instrumentation Available in the Facility

AutoMACS Pro Magnetic Bead separator

Operator: User

Application:

- Single extracellular marker cell sorting
- Depletion/negative cell sorting

gentleMACS Octo Dissociator with Heaters

Operator: User

Application:

- Dissociation of tissues into single cell suspension for culture or flow cytometry assays
- Homogenizes tissues for downstream molecular biology applications

C1 Single Cell Auto Prep System

Operator: User or Staff

Application:

Uses microfluidics, to separate cells into individual compartments, isolate RNA from the single cells, and generate cDNA for downstream genomic applications.

Downstream applications:

- RNA seq
- DNA seq
- PCR

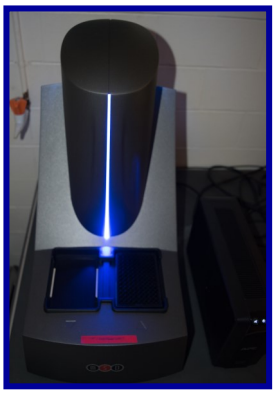
Format: 96 or 384 chambers per chip

MSD Multi-Array Platform

Operator: User

Applications:

- Detection of cytokines, cell signaling proteins
- Multiplexed assay design: (1-10 analytes/plate)
- Detection range: 1 – 10,000 pg/ml
- Sample volumes: 25 µl or less
- Assay Time: 4—6 hours depending on analytes being detected



Nanosight NS 300

Operator: User or Staff

Application:

- Determines the size and concentration of particles 10 nm to 1 microns in size
- Equipped with 4 lasers (405 nm, 488 nm, 532 and 642) to detect fluorescently labeled particles

Zetasizer Nano Z

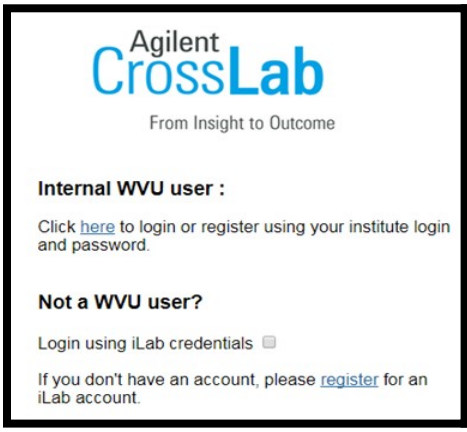
Operator: User or Staff

Application:

Measures the zeta potential of particles in a solution using laser Doppler micro-electrophoresis

Fee Schedule

Instrument	Operator	For WVU & NIOSH Users	For Non-WVU Users
AutoMACS Pro	Facility Staff or User	$4.50 / separation	$6.85 / separation
C1 Single Cell Auto Prep System	Facility Staff	$210/plate	$320/plate
	User	No Cost	$115/plate
FACSAria III	Analysis: Facility Staff	$52.50/h	$80/h
	Analysis: User	$34.65/h	$53/h
	Sorting	$77.70/h	$120/h
	Sorting Setup	$19.43/sort	$30/sort
gentleMACS	Facility Staff or User	$10.50/sample	$16/sample
LSRFortessa	Facility Staff	$52.50/h	$80/h
	User	$34.65/h	$53/h
MSD QuickPlex SQ120	Facility Staff or User	$10.50/h	$16/h
NanoSight NS300	Facility Staff	$61.00/h	$93/h
	User	$42.50/h	$65/h
Zetasizer Nano Z	Facility Staff	$25/sample + $52.50/h	$39/sample + $80/h
	User	$25/sample	$39/sample +



The facility uses iLAB scheduling/billing software from Agilent to manage the use of the facility's instrumentation. If you would like to use the instruments housed in the facility please use the URL shown below to register as a WVU User and to login to reserve an instrument.

https://wvu.corefacilities.org/account/login