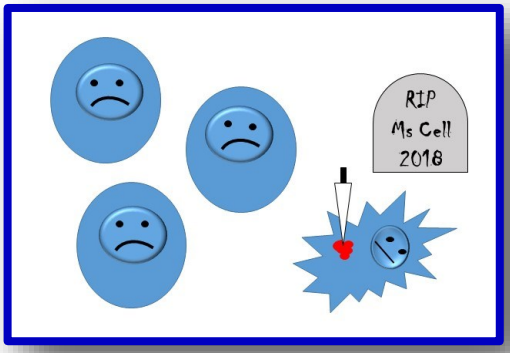
WVU FLOW CYTOMETRY & SINGLE CELL CORE FACILITY

Newsletter Volume 4, issue 3 April 2018

Cell Viability Dyes: Why Use Them and Which One to Use?



In flow cytometry, one of the worst problems to have is a lot of dead/dying cells in your sample. Dead/dying cells can wreck havoc with your flow cytometry experiments due to their tendency to cause cells to aggregate, bind antibodies non-specifically and contribute to cellular autofluorescence. They can be quite a nuisance especially when analyzing rare cell populations or low expression antigens.

Viability dyes aka live/dead stains are commonly used in flow cytometry experiments to identify the dead/dying cells and remove them from the data analysis. These dyes come in a variety of fluorescent colors making it easy to pick one for your experiments that will not interfere with your fluorescently labeled antibodies. Some of these dyes can be used when fixing the cells. Other viability dyes can only be used when cells are not going to be fixed. In the sections below, is a discussion of the different types of viability dyes, how they work and when to use them.

How do viability dyes work?

Broadly, viability dyes can be classified into three different groups based on there mode of action: amine reactive, DNA binding and enzyme activated.

Amine Reactive Dyes

This group of viability dyes react with amine groups on proteins. Like the DNA binding dyes, the amine reactive dyes only get into cells whose membrane has been damaged. By design they don't bind to most surface proteins but do bind to many intracellular proteins. Unlike DNA binding dyes, the amine reactive dyes can be used in protocols requiring cell fixation or permeabilization. When using an amine reactive dye, dead/dying cells will fluoresce brightly while live cells will be dim or not fluoresce at all. Some examples of amine reactive dyes can be found in Table 1.

DNA Binding Dyes

DNA binding dyes have been around for a long time and for the most part are easy to use and inexpensive. With these dyes, the dye will only enter a cell if the membrane has been damaged. Once inside the cell, the dye binds to the cells DNA and in some cases RNA as well. Thus, dead/dying cells will fluoresce while live cells will not. Since these dyes can

Inside this Issue

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2160 HSCN

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email:
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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:
kbrundage@hsc.wvu.edu

Table 1. Amine Reactive Dyes					
Dye Name	Excitation (nm)	Fortessa Laser (nm)	Emission (nm)	Fortessa Detector	Can be used with fixation?
Horizon FVS600	649	628	660	C (APC)	Yes
Live/Dead Green	495	488	520	C (FITC)	Yes
Live or Dye 488/515	488	488	515	C (FITC)	Yes
VivaFix 408/512	408	405	512	A (V500)	Yes
Zombie Violet	400	405	423	B (PacBlu)	Yes

enter a cell through a damaged membrane, these dyes can not be used with cells that will be fixed or are already fixed. Examples of these dyes can be found in Table 2.

Table 2. DNA Binding Dyes					
Dye Name	Excitation (nm)	Fortessa Laser (nm)	Emission (nm)	Fortessa Detector	Can be used with fixation?
7-AAD	543	488	647	A (PerCP-Cy5.5)	No
DAPI	358	405	461	B (PacBlu)	No
Ethidium Monoazide Bromide	504	488	600	B (PI)	No
Propidium Iodide	488	488	617	B (PI)	No
Sytox Blue	440	488	480	C (FITC)	No

Enzyme Activated Dyes

The big difference between enzyme activated dyes and the other two groups of dyes is that enzyme activated dyes stain live cells not the dead/dying cells. These dyes passively cross the cell membrane. Once inside a live cell, the dye is cleaved into a fluorescent form and become membrane impermeant. In addition, a cell can pass the activated dye to its' daughter cells. Like the DNA binding dyes, the enzyme activated dyes can not be used with fixation. Table 3 shows some examples of enzyme activated dyes.

Table 3. Enzyme Activated Dyes					
Dye Name	Excitation (nm)	Fortessa Laser (nm)	Emission (nm)	Fortessa Detector	Can be used with fixation?
Calcein AM	495	488	515	C (FITC)	Yes
Calcein Violet AM	400	405	452	B (PacBlu)	Yes
Cell Tracker CM-DiI	553	561	570	E (PE)	Yes
5-CFDA AM	492	488	514	C (FITC)	Yes
Resazurin/Resorufin	530-560	561	590	D (PE-Texas Red)	Yes

When should a viability dye be used?

You can include a viability dye in any flow cytometry experiment you perform. It is highly recommended to use a viability dye if the cells come from a tissue that has undergone a digestion step to make a single cell suspension and/or red blood cell lysis. Both of these situations will result in cell death/debris that could interfere with your flow cytometry assay. An example is shown on page 6.

If you would like to learn more about viability dyes and assistance with picking the right one for your experiments please contact the WVU Flow Cytometry & Single Cell Core Facility staff for assistance.

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)
- 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 12 fluorochromes

Applications:

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

Other Instrumentation Available in the Facility

AutoMACS 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

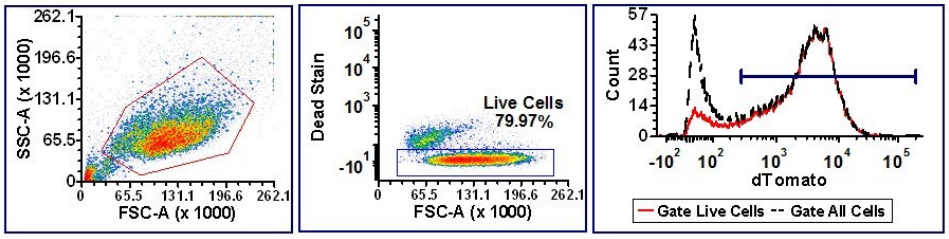
Fee Schedule

Instrument	Operator	For WVU & NIOSH Users	For Non-WVU Users
AutoMACS	Facility Staff or User	$14.70/h	$22.05/h
C1 Single Cell Auto Prep System	Facility Staff	$210/plate	$315/plate
	User	No Cost	$112.50/plate
FACSAria III	Analysis: Facility Staff	$52.50/h	$78.75/h
	Analysis: User	$34.65/h	$51/98/h
	Sorting	$77.70/h	$116.55/h
	Sorting Setup	$19.43/sort	$29.15/sort
gentleMACS	Facility Staff or User	$10.50/sample	$15.75/sample
LSRFortessa	Facility Staff	$52.50/h	$78.75/h
	User	$34.65/h	$51.98/h
MSD Sector Imager	Facility Staff or User	$10.50/h	$15.75/h
NanoSight NS300	Facility Staff	$61.00/h	$91.50/h
	User	$42.50/h	$63.75/h
Zetasizer Nano Z	Facility Staff	$25/sample + $52.50/h	$38/sample + $78.75/h
	User	$25/sample	$38/sample + $15/h

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

FCS Express 6: Gating Out Dead/Dying Cells



Above is an example of how to gate out the dead/dying cells using an amine reactive viability dye. The plot on the left shows the whole sample with a red gate around the cells. The middle density plot is gated on the red cell gate shown in the left dot plot. The gate in this plot is on live cells. The right histogram shows the dTomato expression of the same data gated on either ALL cells (Black line) or LIVE cells (Red line). Depending on the gating, the percent of dTomato (+) is either 72.71% for ALL cells vs 87.26% for LIVE cells. The median fluorescent intensity (MFI) is also different 3300.42 (ALL cells) vs 3449.33 MFI (LIVE cells).

Upcoming Holidays & Events

April 23, 2018 April 26—May 7, 2018	Kathy out of the lab	Facility is open but no sorting or staff assistance
May 8, 2018	Primary Election Day	Facility Closed
May 14 –15, 2018	Kathy out of the lab	Facility is open but no sorting or staff assistance
May 28, 2018	Memorial Day	Facility Closed
July 4, 2018	Independence Day	Facility Closed

New User Guide

Hands-on training for FACSCaliber, 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 grants that support the WVU Flow Cytometry & Single Cell Core in all your publications. The grant numbers are listed below:

MBRCC CoBRE grant: GM103488/RR032138

WVCTS grant: GM104942

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