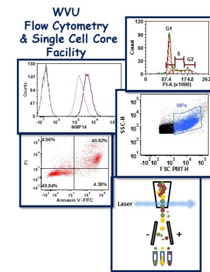
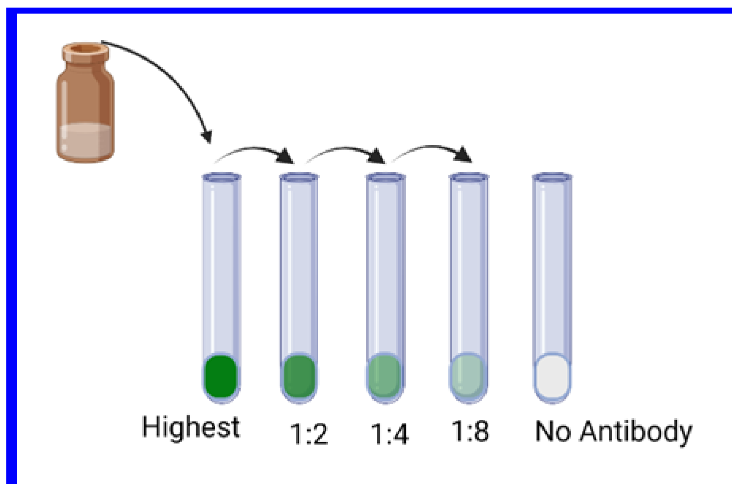


WVU FLOW CYTOMETRY & SINGLE CELL CORE FACILITY

Newsletter Volume 11, issue 1 July 2023

Antibody Titrations: Waste of Time or a Good Practice that can Save Money and Make Data Analysis Easier?



One of the most common mistakes individuals make when doing flow cytometry is they don't take the time to titer the antibodies/fluorescent dyes in their staining panels. Titrating antibodies is very simple process and a great way to become familiar with the staining protocol, operating a flow cytometer, and data analysis. In addition, you will also generate useful information that will make your "real" experiments easier to perform and make the data generated easier to analyze.

What are the benefits of performing antibody titrations?

When you purchase antigen specific antibodies, some companies provide a suggested antibody concentration for a given number of targets (usually 1 x 10^6 cells) in a specific volume (usually 100µl). Other companies provide a range of concentrations, and some don't provide any suggested concentration. Regardless what information is provided by the company there are many reasons why, you should determine the concentration of antibody to use with your cells. Some of the reasons are listed below:

- Antigen densities can vary from cell type to cell type
- Too much excess antibody can cause high backgrounds and increase compensation/spectral

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Facility Location:
Main Lab: 2160 HSCN
Annex Lab: 2184 HSCN
Phone:
304-293-6273
email:
flowcore@hsc.wvu.edu

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

overlaps

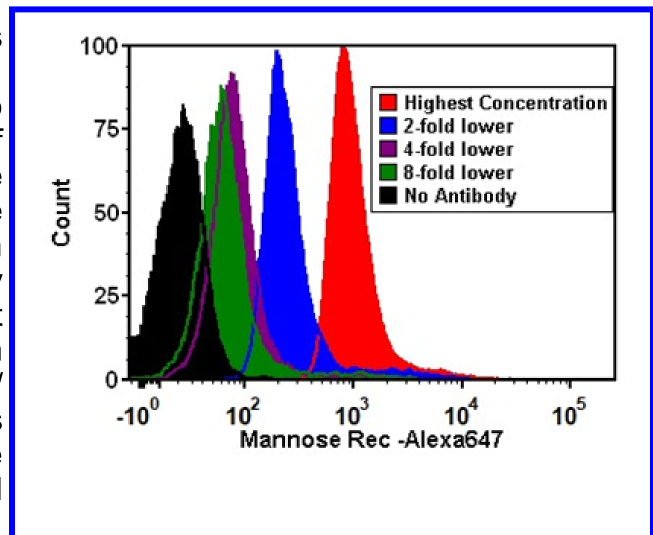
- Using the incorrect amount of antibody can make data analysis difficult
- Minimizes non-specific binding
- Signal will be on scale
- Increases the ability to distinguish dim, normal, and high expressing populations
- Saves money because many times you can use less antibody than what the manufacturer recommends

How do you perform an antibody titration?

Antibody titrations are very easy to perform. It is important to do the titration on the same cell population(s) that you will be using in your “real” experiment. Use the information provided on the technical data sheet provided by the manufacturer as a starting point for the titration.

- ♦ If the company provides a recommended concentration to use, start your titration at that concentration and do a series of 2-fold dilutions down from there. Usually 4 or 5 dilutions is enough.
- ♦ If the company gives you a range, start with the highest concentration and titer down from there, make sure your set of dilutions cover the whole range.
- ♦ If the company does not provide any suggested concentration of antibody to use, start with 1 μg/sample and do a series of 2-fold dilutions down from there.

Once the cells are stained, fix them if that is what you will do in your “real” experiment. On the flow cytometer, set up a FSC vs SSC plot to gate the cells, then set up a series of histograms, one for each antibody in the titration. Run the samples as usual, using the no stain to set the voltages. Once you have run all the samples, determine the optimal antibody dilution. The optimal dilution will be the lowest dilution that resolves positive signal from background and has the best MFI (mean/ medium fluorescent intensity). The MFI values are found in the statistics box. You can use the calculations below to help determine the optimal dilution:



Basic = MFI Positive Population/MFI Negative Population

Staining Index = $\frac{\text{MFI Positive Population - MFI Negative Population}}{\text{2 x robust Standard Deviation (rSD) Negative Population}}$

Separation Index = $\frac{\text{MFI Positive Population - MFI Negative Population}}{(84^{th}\text{ Percentile - MFI Negative})/0.995}$

You can download a titration template that you can use in the FCS Express 7 software at the De Novo website.

When is it appropriate to titrate an antibody?

It is obvious that you should titer any antibody that you have not used before but there are other times when an antibody titration is recommended. Best practice recommends

performing an antibody titration when
- ◊ Using the antibody in a new assay
- ◊ Changing antibody lots
- ◊ Using a different antibody clone
- ◊ The antibody is conjugated to a different fluorochrome
- ◊ Changes have been made to the protocol

If an antibody titration was performed on one instrument, can you use the same titer on another?

The answer to the above question is yes for the Fortessa and ARIA and maybe on the Aurora.

Why maybe?

Because, the Fortessa and Aurora use different types of detectors and the way we adjust the sensitivity of the detectors is quite different. On the Fortessa, detectors are adjusted individually so you can increase one, decrease another without affecting the signal strength of the different fluorochromes. Whereas with the Aurora all detectors associated with the laser are adjusted by increasing or decreasing the gain by the same percentage regardless of the optimal detector for a particular fluorochrome.

In conclusion, you can probably use the same titer on both the Fortessa and Aurora. However, if there is a live/dead stain in your panel it is highly recommended that you check/titer the live/dead stain again if the original titer was determined on the Fortessa.

Why re-titer the live/dead stain?

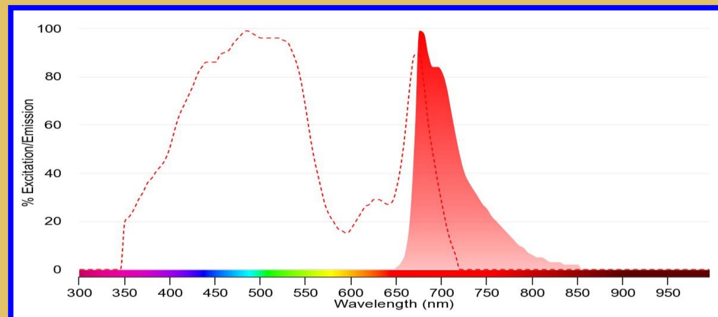
Due to the higher sensitivity of the detectors in the Aurora, how bright most live/dead stains are, and the fact that if you have to decrease the gains by a percentage across the detectors to get the live/dead signal on scale you may inadvertently lose detection of dim signals.
For more information on how to determine the best concentrations for the antibodies in your staining panel contact the WVU Flow Cytometry & Single Cell Core Facility staff.

Highlighted Fluorochrome: PerCP-Cy5.5

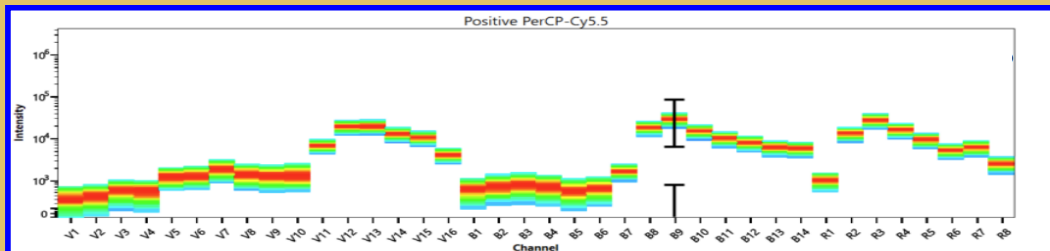
PerCP-Cy5.5 is a tandem dye that is fixative and is excited by the 488 nm blue laser, making it a versatile addition to panels.

Excitation: 482 nm

Emission: 690 nm



Traditional spectrum as seen on the Fortessa and ARIA



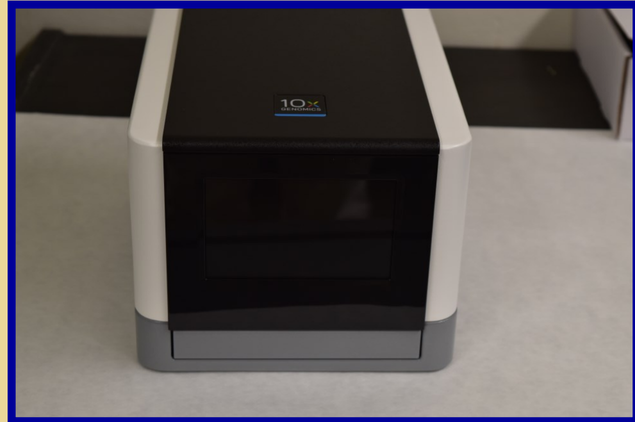
Spectral signature as seen on the Aurora

10x Genomics Chromium Controller

Operator: Facility Staff

Applications:

- Single cell barcoding for 500 to 10,000 cells
- Whole cell or nuclei samples
- Gene Expression Analysis
- Immune Profiling
- ATAC
- Multiome ATAC + Gene Expression
- Spatial Gene Expression



10x Genomics Chromium Controller Fees

	For WVU Users	For Non-WVU Users
Instrument Use	$175	$290
Chip*	$260	$430
Reagents	$50/sample	$85/sample
cDNA Tracer-tape	$9/sample	$14/sample
Multiome/ATAC Sample Prep	$225/cell line sample	$350/cell line sample
	$270/frozen tissue	$420/frozen tissue
Labor**	$50/hour	$83/hour

*Holds up to 8 samples

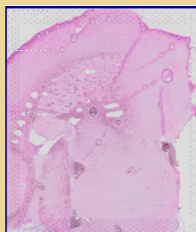
**The GEM generation & Barcoding takes about 2 – 2.5 hours. The generation of the library can take 3.5 – 10 hours depending

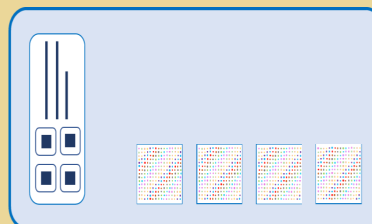
10x Genomics Visium Transcriptomics

Operator: Facility Staff

Applications:

- Gene expression mapping on tissue
- Fresh-frozen or FFPE tissues
- Analysis of whole transcriptome within select tissue section





10x Genomics Visium Transcriptomics Fees

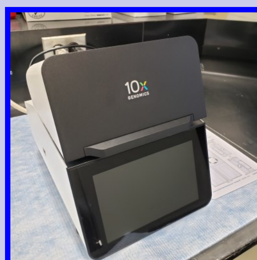
	For WVU Users	For Non-WVU Users
Optimization Slide	$350	$540
Reagents for spatial gene expression	$60/sample (4 samples per slide)	$93/sample (4 samples per slide)
Labor for spatial gene expression	$50/hour (process takes approximately 12 hours)	$78/hour (process takes approximately 12 hours)

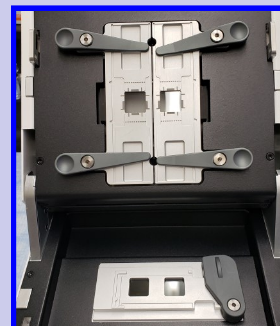
Spatial Transcriptomics with 10x Genomics CytAssist

Operator: Facility Staff

Applications:

- Simplifies Visium workflow
- Fresh-frozen or FFPE tissues
- Analysis of whole transcriptome of whole tissue section





Spatial Transcriptomics with CytAssist Fees

	For WVU Users	For Non-WVU Users
Instrument Usage	$100/slide	$155/slide
Reagents for spatial transcriptomics	$60/sample (2 samples per slide)	$93/sample (2 samples per slide)
Labor for spatial transcriptomics	$50/hour	$78/hour

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

Cytek Aurora Full Spectrum Flow Cytometer

Operator: User or Facility Staff

Three lasers:
- 405 nm Solid State violet
- 488 nm Solid State blue
- 640 nm Solid State red

Twenty-seven parameter analysis:
- Forward Scatter on blue laser
- Side Scatter on blue laser and violet laser
- 24 different fluorochromes

Applications:

Flow Cytometers in the Facility (continued)

LSR Fortessa

Operator: User or 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

Guava easyCyte HT

Operator: User or Facility Staff

Lasers:
- 488 nm solid state

Detection Parameters:
- Forward Scatter
- Side Scatter
- Simultaneous detection of 3 fluorochromes

Applications:
- Cell Counts
- Apoptosis Assay
- Cell Cycle Analysis

Other Instrumentation Available in the Facility

MultiMACS Cell24 Separator Plus

Operator: User
Application:
- High throughout manual separations
- 1-24 samples
- Positive and negative cell separation

gentleMACS Octo Dissociator with Heaters

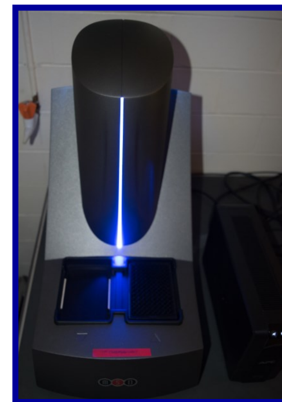
Operator: User
Application:
- Tissue disassociation into single cell suspension for culture or flow cytometry assays
- Tissue homogenization for molecular biology applications

AutoMACS Pro Magnetic Bead separator

Operator: User
Application:
- Single extracellular marker cell sorting
- Depletion/negative cell sorting

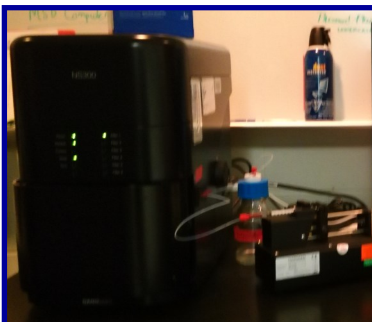
MSD Multi-Array Platform

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
Aurora	Facility Staff	$52.50/h	$80/h
	User	$34.65/h	$53/h
AutoMACS Pro	Facility Staff or User	$4.50 / separation	$6.85 / separation
FACSAria III	Analysis: Facility Staff	$52.50/h	$80/h
	Sorting	$77.70/h	$120/h
	Sorting Setup	$19.43/sort	$30/sort
gentleMACS	Facility Staff or User	$10.50/sample	$16/sample
Guava easyCyte	Facility Staff	$52.50/h	$80/h
	User	$34.65/h	$53/h
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
MultiMACS Cell24 Separator Plus	Facility Staff or User	$3/separation	$4.65/ separation
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 + $16/h

Upcoming Holidays & Events

August 8-14, 2023	Kathy out of lab	Facility Open and All Services Available Except 10x Genomics
September 4, 2023	Labor Day	Facility Closed
November 22-24, 2023	Thanksgiving Break	Facility Closed
December 22-26, 2023	Christmas Break	Facility Closed

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

WV InBRE grant: GM103434

WVCTS grant: GM104942

Aurora S10 grant: OD028605

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

New User Guide

Hands-on training for LSRFortessa, Cytek Aurora, 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.

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/landing/984