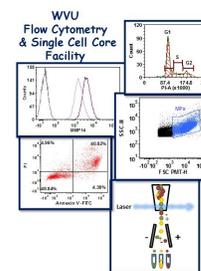


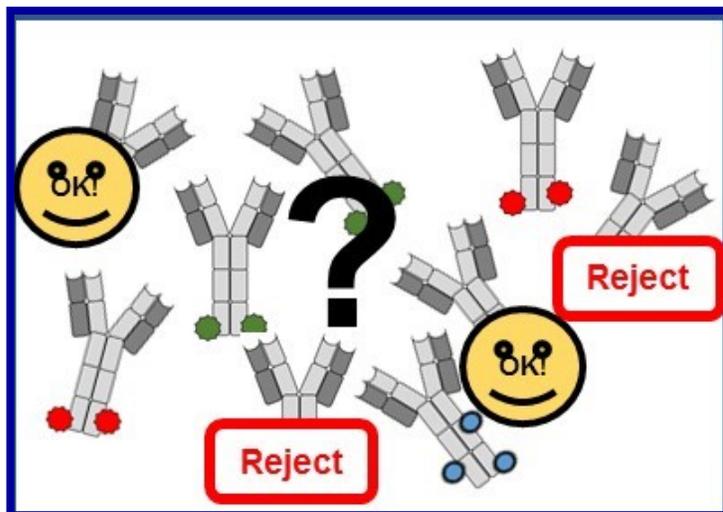
WVU FLOW CYTOMETRY & SINGLE CELL CORE FACILITY



Newsletter Volume 5, issue 1

July 2018

Finding and Validating Antibodies Part 1



One of the most frequent questions that the WVU Flow Cytometry & Single Cell Core Facility gets asked is “What antibody should I use to detect protein of interest and where do you recommend buying it from?”. As we all know there are many reputable companies out there that have really good antibodies but unfortunately there are some companies whose antibodies do not live up to expectations. So, the real question is how do you know the antibody you purchase is specific for protein of interest and will work as advertised?

A few years ago, NIH began to specifically request that investigators put in their grant applications a plan for the authentication of key biological and chemical reagents that will be used in the proposed research. NIH considers antibodies to be key biological reagents. This leads to another frequently asked question: How do you authenticate/validate antibodies?

In the last few years, a number of papers have been published which discuss how to go about finding the correct antibody and validating it. Below are three examples of such papers:

1. Bordeaux, J., et al. 2010. Antibody Validation. *Biotechniques*. 48(3): 197-209.
2. Roncador, G., et al. 2015. The European Antibody Network’s Practical Guide to Finding and Validating Suitable Antibodies for Research. *mAbs*. 8(1): 27-36.
3. Weller, M.G. 2018. Ten Basic Rules of Antibody Validation. *Analytical Chemistry Insights*. 13:1-5.

In this issue of the newsletter as well as the next, we will outline how to go about finding a good antibody that works in your assay in a reproducible manner. Think of the antibody validation as a four step process (See flowchart on right side of this page). The first step is to

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Facility Location:
2160 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

define the antigen and assay requirements. The second step is to collect as much information from the literature, antibody websites and companies. The third step is to look at what validation already has been done on the antibody. Finally the last step is to demonstrate in your lab that you get the same results as other labs.

Step 1: Define the antigen requirement

This step may seem like a no brainer but you would be surprised at the number of people who don't think about the antigen and its properties prior to picking an antibody. In this step, the target antigen is identified, the antibody source (monoclonal vs polyclonal) is determined and the assay that the antibody will be used in is chosen.

Identifying the antigen is more than just determining that you want an antibody to detect X. Identifying the antigen means you need to know something about protein of interest such as: Does it come in multiple forms (alternative spliced variants, phosphorylated and unphosphorylated)? If yes, then which form do you want to detect or do you want to detect all forms? Is the antigen expressed on the surface or intracellular? If it is an intracellular protein where is it found? Cytoplasm? Nucleus? These are just some of the questions you need answer to prior to selecting an antibody.

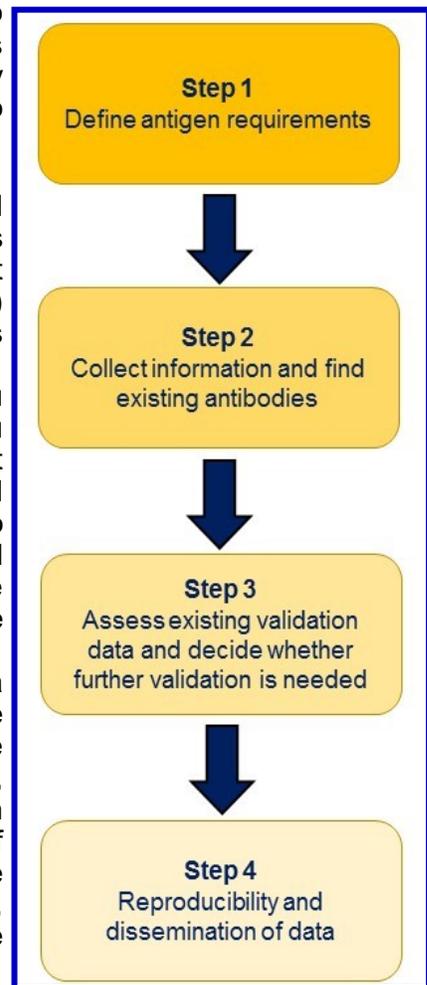
Identifying the antibody source at this step relates to choosing a monoclonal vs a polyclonal antibody. Monoclonal antibodies are usually the first choice for several reasons: they detect a single antigen epitope so there should be less chance of cross-reactivity, the supply is endless, as long as the cell line is not lost, and batch to batch variability is limited. Polyclonal antibodies are a mixture of antibodies recognizing multiple epitopes which can increase the chance of cross reactivity and background in the assay. In addition, the supply of antibody is limited to the life of the animal so over time the antibody milieu will change.

Finally, knowing what assay you will be using the antibody in is important because different assays will have different antibody requirements. For example in flow cytometry, antibodies have to recognize proteins in their native folded state so antibodies that are generated by immunization with a peptide may not work. Conversely for western blots, an antibody generated using a peptide should work very well, while an antibody generated by immunization with the native protein may not. Remember, one antibody does not necessary fit all assays. In fact using more than one antibody to detect the same protein is a good way to demonstrate that the results you obtained are reproducible and related to the protein being studied and not the antibody used in the assay.

Step 2: Collecting information and finding existing antibodies

For this step the idea is to find out what antibodies are available to detect protein of interest. First, check the literature. Look to see what antibodies have been used by others to detect protein of interest in the assay you are planning to use it in. Check antibody websites such as Linscott's Directory (www.linscottsdirectory.com) or The Antibody Resource Page (www.antibodyresource.com) to find companies that sell antibodies to protein of interest. Once you have a list of antibodies, go to the company's website and pull up the antibody datasheet. On the datasheet it should tell you which assays the antibody has been tested in, what was used as the immunogen to produce the antibody and what conjugated forms it is available in. If you are looking at monoclonal antibodies the name of the cell line (clone) that produces the antibody should be noted. In many cases, the same cell line/clone is used by multiple companies with each company having different conjugated forms.

Once you have your list of available antibodies you are ready to move on to Step 3. In the next issue of the newsletter, we will finish the discussion on finding and validating antibodies by going over Step 3 & 4 of the flow chart.



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 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



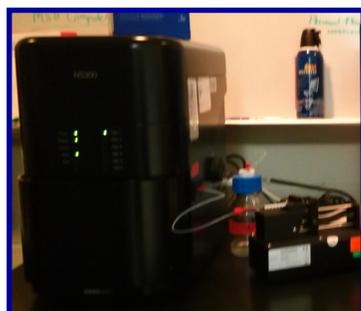
Nanosight NS 300

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	\$14.70/h	\$22.05/h
C1 Single Cell Auto Prep System	Facility Staff	\$210/plate	\$315/plate
	User	No Cost	\$112.50/plate
FACS Aria 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 +	\$38/sample +
	User	\$25/sample	\$38/sample +



From Insight to Outcome

Internal WVU user :

Click [here](#) to login or register using your institute login and password.

Not a WVU user?

Login using iLab credentials

If you don't have an account, please [register](#) for an iLab account.

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: Converting a Marker into a Gate

Some times when performing data analysis you may want to further analyze a subpopulation that you have identified on a histogram with a marker. When FCS Express markers are drawn they are not gates but they can be converted to gates in a few easy steps.

1. Right click on the graph with the marker you want to convert into a gate.
2. From the drop down menu that appears, click on "Convert Marker to Gate"
3. A menu opens, Click on "Convert"
4. A "Create New Gate " Box opens. Choice the following options
 - A. Either "Create a New Gate" or "Replace an Existing Gate"
 - B. Give the gate a name
 - C. Select a color for the gate
 - D. Choose to either include or exclude the events in the gate
 - E. If you want, select "Choose to emphasize on plots"
 - F. Click "Okay"

Now the marker is a gate and you can use it like any polygon or rectangle gate drawn on 2 parameter graphs.

Upcoming Holidays & Events

July 4, 2018	Fourth of July	Facility Closed
July 18 –23, 2018	Kathy out of the lab	Facility is open but no sorting or
September 3, 2018	Labor 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 FACS Aria 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 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