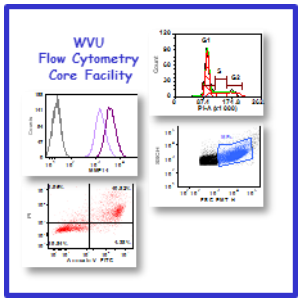
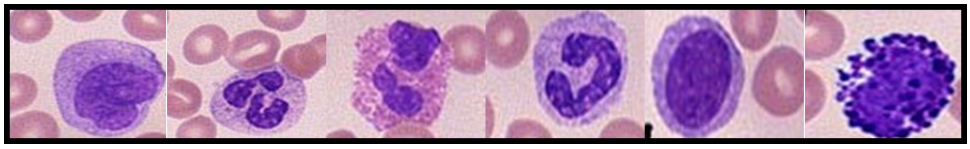
WVU FLOW CYTOMETRY CORE FACILITY



Newsletter Volume 2, issue 2 October 2015

Assay Highlight: Collecting, Storing and Processing Human Blood



Often researchers come to the Flow Cytometry Core Facility to ask how they should collect/store/process human blood samples. In this issue of the newsletter, we discuss the best practice on human blood collection, storage and processing.

As with many things, there is no one size fits all when handing human blood samples. Many assays have very specific requirements for the blood collection, processing and storage. However, certain general rules should be followed regardless of assay. These include:

1. Blood samples should be processed as soon as possible
2. For most assays, keep the blood sample at room temperature.
3. Maintain the blood sample as aseptically as possible
4. Gently rocking of the blood sample will prevent cell aggregates
5. Use universal precautions when handling human blood cells

The first thing to consider when using primary human blood cells is which anti-coagulant is appropriate for the cell type you are interested in studying. The choice of anti-coagulant is highly dependent on the cell type of interest and the assay the cells will be used for. The table to the left lists commonly performed assays and the most appropriate anti-coagulant for blood collection. The table also shows how long after collection the cells may be stored (at room temperature) before performing the assay.

Assay	Anti-Coagulant	Storage Time
Lymphocyte Phenotyping	Sodium Heparin or EDTA	≤ 72h
Myeloid Phenotyping	EDTA	Use Immediately
Neutrophil Function	Sodium Heparin or EDTA	Use Immediately
Platelet Activation	EDTA	Use Immediately
Platelet Phenotyping	EDTA	Use Immediately
DNA Analysis	Sodium Heparin or EDTA	Use Immediately

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Facility Location:
2160 HSCN
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e-mail: flowcore@hsc.wvu.edu

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

After hours access for experienced users by prior approval from Kathy Brundage

Contact Kathy at:

kbrundage@hsc.wvu.edu

Although some assays can be performed using whole blood, in most assays removal of the red blood cells (RBCs) is desirable. There are two common methods for removing RBCs: lysis and density gradient centrifugation. RBC lysis will yield more cells than density gradients. There are a number of red blood cell lysis buffers available commercially. Lysing RBCs by osmotic shock using ammonium chloride works just as well. One thing to keep in mind when using commercial RBCs lysis reagents is that some of these reagents contain a fixative as well. Regardless of which method is used to remove the RBCs, it is important to check how the process affects the cell population(s) of interest and the downstream assay.

In some instances storing the cells for later use is necessary. The main way to store the cells long term is cryopreservation. If you are going to cryopreserve the cells, it is best to process the blood sample remove the red blood cells (RBCs) by lysis or density gradient centrifugation. Once you have done that, it is possible to use a standard cell freezing protocol with DMSO to cryopreserve the white blood cells. If you do decide to cryopreserve your cells keep in mind the following issues:

1. Expression of some antigens may be affected by cryopreservation
2. Some cell populations don't come out of cryopreservation well and can be lost
3. When thawed there may be more cell death than observed in fresh samples

One way to improve cell recovery after cryopreservation is to incubate the cells in tissue culture medium at 37°C for a few hours prior to staining them or placing them into an assay.

In addition to cryopreservation, there are two commercially available storage reagents that will preserve whole human blood for later use in flow cytometry assays. These two reagents are Transfix (ThermoFisher Scientific, Waltham MA) and Cyto-Chex (Streck, Omaha NE). As with cryopreservation, it is recommended that you test how these storage reagents affect the cell population(s) of interest in your assay.

For more information and specific protocol on the collection, processing and storage of human blood samples you should read Chapter 5 of Current Protocols in Cytometry. A copy of which can be found in the WVU Flow Cytometry Core Facility.

To log in and reserve a flow cytometer, AutoMACS, MSD or analysis computer, please point your browser to the following URL →

CORES
Core Ordering & Reporting Enterprise System
https://cores-wvu.mis.vanderbilt.edu/login.cfm

Instruments in this facility

FACSAria Cell Sorter/Analyser
Operator: Facility Staff
Lasers: 488 nm Sapphire(SS)
633 nm HeNe
407 nm Violet(SS)
Detection Parameters:
Forward Scatter, Side Scatter, simultaneous detection of up to 11 fluorochromes
Applications:
Cell Sorting (Aseptic)
Cell phenotyping
Cell Viability
FISH, FRET, SPA

FACSCalibur Analyser
Operator: User
Lasers: 488 nm Argon
633 nm Red Diode
Detection Parameters:
Forward Scatter, Side Scatter, simultaneous detection of up to 4 fuorochromes
Applications:
Cell phenotyping
Cell cycle analysis

AutoMACS Magnetic Bead separator
Operator: User
Application:
single marker (extracellular) sorting, depletion sorting

LSR Fortessa
Operator: User
Lasers: 405 nm OBIS LX
488 nm Sapphire (SS)
561 nm Sapphire (SS)
628 nm OEM
Detection Parameters:
Forward Scatter, Side Scatter, simultaneous detection of up to 12 fluorochromes
Applications:
Cell phenotyping
Cell Viability
Cell Cycle analysis
FISH, FRET, SPA

Analysis Tips - NEW FCS Express 5 Software !!!

In early October, an email went out to all WVU Flow Cytometry Core Facility Users announcing an upgrade to the FCS Expression analysis software from version 4 to version 5. FCS Express 5 is very similar to the older version with some new and improved features. One important new feature is an internet dongle which allows users to download the software to their own computer (as long as you have an internet connection) and perform data analysis outside the Flow Cytometry Facility.

In order to take advantage of the internet dongle feature:

1. Contact the Flow Cytometry Core via email (kbrundage@hsc.wvu.edu or flowcore@hsc.wvu.edu)
2. You will be sent a user login, password , configuration file and step by step downloading instructions

In addition, we have set up a series of webinars on how to get the most out of the software.

October 29 (Thursday)	3-4 pm Room 2157	New Features in FCS Express 5
November 19 (Thursday)	3-4pm Room 2157	Using Tokens, Batch Processing, and
January 15 (Friday)	2-3pm Room 2157	Cell Cycle and Proliferation
February 17 (Wednesday)	2–3pm Erma 201	Regression Analysis, Generating Bar

If you have a topic you would like to have discussed please contact the Flow Cytometry Core Facility so that it can be put on the agenda.

Fee Schedule (2015-2016 hourly rates)

	WVU user	Non-WVU user
Data acquisition by User	**$33.00**	**$49.50**
Facility assistance for data acquisition	**$17.00**	**$25.50**
Data acquisition by Facility Staff	**$50.00**	**$75.00**
Sorting	**$74.00**	**$111.00**
Data Analysis (unassisted)	**0.00**	**0.00**
Data Analysis by Facility Staff	**$50.00**	**$75.00**

GOOD NEWS FOR WVU INVESITAGORS WHO WOULD LIKE TO SORT

The HSC Research Office has generously provided the facility with some funds to subsidize/co-pay users' sorting costs. From Sept 2015 through Feb 2016, the cost of sorting for a WVU researcher is $15/hour.

New User Guide

Hands-on training for FACSCaliber and LSRFortessa is **mandatory** for all new users and must be scheduled by consultation with facility director.

Sorting as well as data acquisition on FACSAria is by facility staff only.

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.

Helpful Websites

http://www.antibodyresource.com/superstars from The Antibody Resource

Provides small trial size kits of antibodies from multiple companies that have been reviewed by users and demonstrated to work well in many different assays.

http://www.linscottsdirectory.com/

Allows you to search for antibodies from multiple companies based on reactivity, assay, form etc.

http://www.citeab.com/

A large antibody database that ranks search results based on citations

http://www.labome.com/

Site allows you to search not only for antibodies but siRNA/shRNAs, mircoRNAs and cDNA clones

http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1096-9861/homepage/jcn_antibody_database.htm

From this site you can download an Excel spreadsheet containing antibodies that react specifically with proteins found in neuronal cells along with references

http://1degreebio.org/

A large searchable antibody database that includes user reviews for each antibody

Upcoming Events

October 29	**FCS Express Webinar**	**3 –4 pm Room 2157**
November 19	**FCS Express Webinar**	**3 –4 pm Room 2157**
November 25 - 27	**Thanksgiving Break**	**Facility Closed**
December 24 – 28	**Winter Break**	

Note to users:

Please acknowledge the WVU Flow Cytometry Core Facility when reporting your flow cytometry data, using the appropriate phrase(s):

FACSAria users: Flow Cytometry experiments were performed in the West Virginia University Flow Cytometry Core Facility, which is supported by the National Institutes of Health equipment grant number RR020866 and the Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant numbers P30GM103488 (CoBRE) and P20GM103434 (INBRE).

LSRFortessa users: Flow Cytometry experiments were performed in the West Virginia University Flow Cytometry Core Facility, which is supported by the National Institutes of Health equipment grant number S10OD016165 and the Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant numbers P30GM103488 (CoBRE) and P20GM103434 (INBRE).