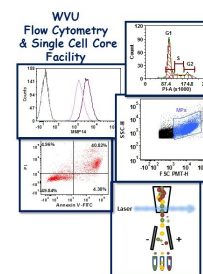


WVU FLOW CYTOMETRY & SINGLE CELL CORE FACILITY



Newsletter Volume 5, issue 3

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How to Measure Autophagy Using Flow Cytometry

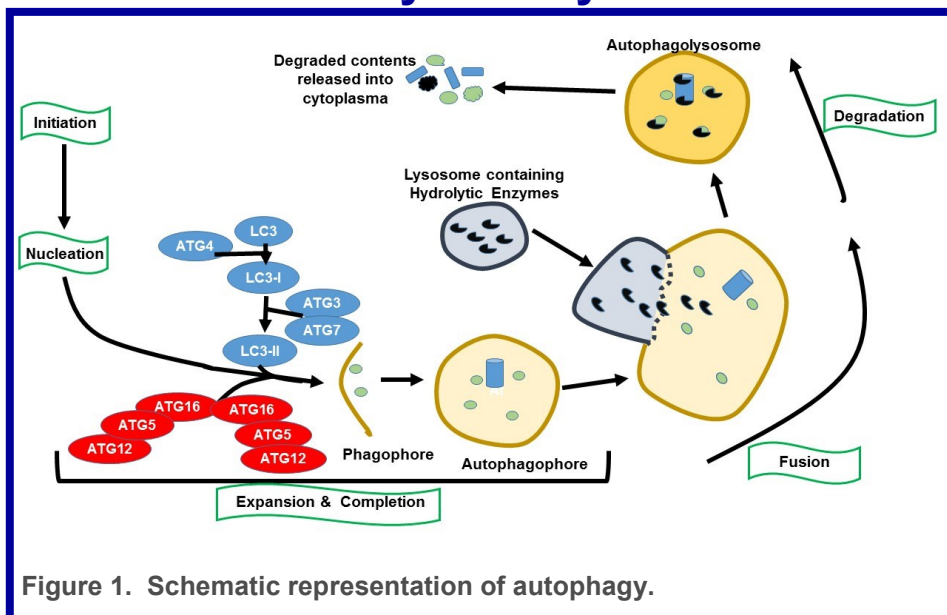


Figure 1. Schematic representation of autophagy.

Autophagy is a normal cellular process involving protein degradation and turnover of organelles as part of a mature cell's homeostasis process. It allows for the orderly degradation and recycling of cellular components. Autophagy can be activated by many environmental conditions including oxygen and nutrient availability, hormones, cytokines, growth factors and receptor engagement.

Autophagy is also an important part of cellular differentiation. It is part of erythropoiesis, as well as hematopoietic stem cell differentiation from embryonic stem cells and adipocytes or osteoblasts from mesenchymal stem cells. Mature cells that are long lived like cardiomyocytes, skeletal muscle cells, neurons and quiescent immune cells use autophagy to maintain cellular health. Autophagy has also been shown to have a role in a cell's stress response, infections, cancer and neurodegeneration diseases.

Autophagy Process

The autophagy process involves 5 steps/stages (Figure 1). The first stage is the initiation stage. As stated above, there are many factors

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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

that can start the autophagy process, but the common step in the process is the activation of mTOR. Activation of mTOR leads to the activation of Unc-51 like autophagy activating kinase (ULK) and the formation of an ULK complex. Nucleation is the next step, and it involves the formation of the VPS34 complex. The third step in the process is the expansion and completion process. In this step, the autophagy related protein (ATG) complex forms along with the formation of phosphatidylethanolamine conjugated LC3 (LC3-II). The ATG complex directs LC3-II into the phagophore membrane. Eventually the phagophore closes and forms the autophagosome. Step 4 is the fusion step. At this stage the autophagosome fuses with a lysosome. Step 5 is the degradation of the proteins in the autophagolysosome followed by the degraded contents being released into the cytoplasm.

Assaying for Autophagy

Fluorescent microscopy methods were used initially to assess autophagy. More recently, flow cytometry has been used to assay autophagy. The flow cytometry assays involve detecting LC3 (precursor to LC3-I) and LC3-I and LC3-II. There are several commercially available kits that use flow cytometry to assay autophagy (see Table 1). These kits provide researchers with another methodology to assess autophagy.

If you are interested in investigating autophagy in your research model and would like to learn more about how to assay autophagy using flow cytometry stop by the WVU Flow Cytometry & Single Cell Core Facility for a chat. We would be happy to work with you use autophagy detection by flow cytometry in your research project.

Table 1. Flow Cytometry Assays for Autophagy		
Company	Kit Name	How the kit works
Abcam	Autophagy Assay Kit (139484)	Uses a cationic amphiphilic tracer (CAT) dye that rapidly partitions into cells similar to drugs that induce phospholipidosis
Bio-Rad	Autophagy Assay Kit (APO010A)	Uses a cell-permeant aliphatic molecule that fluoresces brightly when inserted into the lipid membranes of autophagosomes and autolysosomes
Enzo	CYTO-ID Autophagy Detection Kit (ENZ-51031-0050)	Uses a cationic amphiphilic tracer (CAT) dye that rapidly partitions into cells similar to drugs that induce phospholipidosis
Millipore Sigma	FlowCollect Autophagy LC3 antibody based Assay Kit (FCCH100171)	Uses anti-LC3 FITC conjugate and autophagy enabling reagents that prevents the lysosomal degradation of LC3
Sigma Aldrich	FlowCollect LC3-GFP Reporter Autophagy Assay Kit (FCCH100170)	Uses a permeabilization solution discriminates between cytosolic LC from autophagic LC3 by extracting the soluble cytosolic proteins. Uses a monomeric GFP as a reporter. The autophagy detection reagent will prevent the lysosomal degradation of LC3.
Sigma Aldrich	FlowCollect Autophagy Detection Reagent Pack (CF200097)	

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	\$4.50 / separation	\$6.85 / separation
C1 Single Cell Auto Prep System	Facility Staff	\$210/plate	\$320/plate
	User	No Cost	\$115/plate
FACS Aria 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
LSR Fortessa	Facility Staff	\$52.50/h	\$80/h
	User	\$34.65/h	\$53/h
MSD Sector Imager	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 + \$16/h



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>

Upcoming Holidays & Events

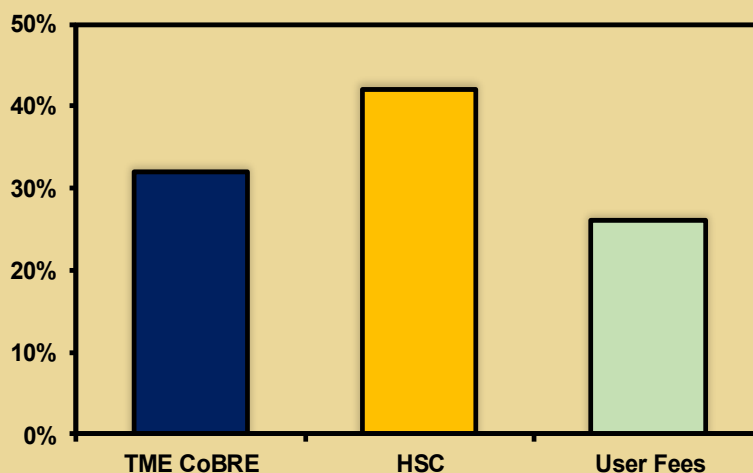
April 19, 2019	Spring Holiday	Facility closed
April 22, 2019	Kathy out of lab	No sorting; Other instruments in facility available for EXPERIENCED users only
May 27, 2019	Memorial Day	Facility closed
June 18 — 27, 2019	Kathy out of lab	No sorting; Other instruments in facility available for EXPERIENCED users only

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.

Revenues sources for WVU Flow Cytometry & Single Cell Core Facility (FY2019)



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