

Flow Cytometry and Immune Monitoring

University of Kentucky
and
Markey Cancer Center

Services and Rates

July 2022 through June 2023

About the Flow Cytometry & Cell Sorting Facility

The Flow Cytometry & Immune monitoring Facility (FCIM) is a shared research facility with a mission to provide technical and scientific support for investigators in the area of immunology research. From simple immunological assays to complex studies involving immunology, FCIM provides access to team of expert immunologists and state of art instruments to aid your pre-clinical, translational, and clinical research activities at the University of Kentucky. FCIM team comprise experts in flow cytometry, tumor immunology, immunotherapy, vaccine biology, inflammation, autoimmune diseases, immune biomarker discovery, drug development and testing. We provide both fee based service and scientific collaboration for research in the area of immunology involving human or animal studies.

Listed below are popular services provided at FCIM. For more information or questions regarding our services please contact FCIM.

Routine FCIM SRF services include:

- **Flow cytometric analysis**
 - Cell phenotyping
 - DNA content and cell cycle
 - Apoptosis and necrosis
 - Cytotoxicity assays
 - Oxidative stress
 - Intracellular cytokine
 - Signal transduction
 - Cell activation
- **Cell sorting**
 - Sterile cell sorting
 - Single cell cloning
- **Magnetic cell sorting**
 - Positive cell isolation
 - Negative cell isolation
 - Rare cell enrichment
- **Immune monitoring**
 - Peripheral blood cell isolation
 - Plasma/serum isolation
 - Cell banking services
 - Cytokine/growth factor analysis
 - ELISA
 - Elispot
 - Luminex
 - Leukocyte functional assays
 - Proliferation
 - Cytokine synthesis
 - Antibody synthesis
 - Cell cytotoxicity
 - Cellular product services
 - T cell expansion
 - Macrophage/DC generation
 - Antibody titers
 - Immune Assay Design Services
 - Research collaborations

Major Instrumentation

iCyt-Sony Cell Sorter System: This flow cytometer system contains two separate sorter units housed in a BSL-2 level biosafety cabinet. Each sorter unit is equipped with 5 lasers (355 nm, 405nm, 488nm, 561nm and 642nm) and can sort or analyze cells labeled with up to 12 fluorescent antibodies or probes. In addition, the biocontainment setup allows sorting and analysis of infectious samples (risk groups 1 and 2) and live human samples. Cells can be sorted sterilely into 5 ml (12 x 75 mm) tubes, 15 ml conical tubes or directly into multiwell plates.

Bigfoot spectral cell sorter: The cell sorter with 5 lasers (349 nm, 405 nm, 488 nm, 561 nm, 640 nm) and 53 detectors utilizes the newest technology of spectral flow cytometry, which captures the entire emission spectrum of each fluorochrome rather than just the peak emission band. This sorter is also housed in a BSL-2 level biosafety cabinet for use with infectious samples (risk groups 1 and 2) and live human samples. This sorter can perform 4-way sorting or can be performed into multiwell plates.

Cytek Aurora: The Aurora is full spectrum flow cytometer with 5 lasers (55 nm, 405 nm, 488 nm, 561 nm, and 640 nm) and 67 detectors. The new technology of spectral flow cytometry greatly increases the capacity for multi-dimensional analysis in complex cell populations.

BD FACSymphony (units #1 and #2): The FACSymphony cell analyzers are equipped with 5 lasers and 23 detectors. It can simultaneously measure up to 21 fluorescent antibodies or probes, as well as forward light scatter (cell size) and side light scatter (cell granularity).

CytoFLEX LX (Beckman Coulter): This cell analyzer is equipped with 5 lasers: blue, red, violet, yellow, and near UV and can simultaneously measure up to 19 fluorescent antibodies or probes, as well as forward light scatter (cell size) and side light scatter (cell granularity). It is fitted with plate loader for 96-well plates for analysis, can analyze sample volumes as low as 10 ul and has nanoparticle resolution as low as 200 nm. It also performs absolute cell counts for every sample and population.

MACSQuant VYB (Miltenyi Biotec): This cell analyzer is fitted with 3 lasers (405nm, 488nm and 561nm) which allows simultaneous measurement of up to 8 fluorescent antibodies or probes, as well as forward light scatter (cell size) and side light scatter (cell granularity). This instrument is a fully automated multi-color flow cytometer that performs automated staining of cell samples and automated cell analysis.

AutoMACS Pro Magnetic Cell Separator: This cell sorter is a high performance immunomagnetic cell separation instrument with fully automated functions for hands-free operation, such as fully automated labeling of multiple samples. Sorting on this instrument utilizes antibodies that are labeled with an iron-containing compound, which are then separated automatically from unlabeled cells with a rare earth magnet. Cells can be separated using positive selection, depletion or untouched isolation programs. Cooling racks allow the maintenance of integrity of samples and eluted fractions. This magnetic cell sorter is useful for pre-enrichment prior to cell sorting or analysis, especially when rare cells are of interest.

ImmunoSpot S6 Universal Visible/Fluorescent Analyzer: This instrument provides high-resolution visible and fluorescent light analysis for use in applications that benefit from morphometric analysis, including ELISPOT assays, colony counting (bacterial, yeast, stem cell, tumor cells), plaque and clonogenic assays, and genotoxic/cytotoxic assays. It is compatible with all plate formats from 100mm Petri dish to 384-well microplates.

Data Analysis: Standardized data analysis is available to investigators for each set of samples. Data can be reanalyzed at the request of the investigator with a 30-minute minimum service charge. The flow facility also makes available several workstations and software options for users to analyze their own data at no additional charge. The facility also has a site license for FlowJo software. Investigators upon request can purchase annual licenses for FlowJo.

Locations and contact info: Flow Cytometry and Cell Sorting Lab:
MS408 Willard Medical Education Building
Telephone: 323-6859

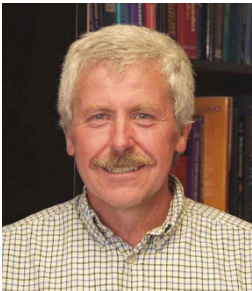


Immune Monitoring Lab:
MS410 Willard Medical Education Building
Telephone: 323-5584

Website: <https://www.research.uky.edu/flow-cytometry-and-immune-monitoring-core-facility>

Biosafety Questionnaire: <https://www.research.uky.edu/uploads/flow-cytometry-biosafety-questionnaire>

Sample Information Sheet:

<https://www.research.uky.edu/uploads/flow-cytometry-sample-info-form>

FCIM team	
 <p>Don Cohen, PhD Facility Director Contact: 859-323-5131 Email: dcohen@uky.edu</p>	 <p>Jerry Woodward, PhD Facility Co-Director Contact: 859-323-5538 Email: jwood1@uky.edu</p>
 <p>Siva Gandhapudi, PhD IM lab manager Contact: 859-323-5584 Email: skgand2@uky.edu</p>	 <p>Jennifer Strange Flow lab manager Contact: 859-323-6859 Email: jstra1@uky.edu</p>

Flow Cytometry Service Rates for 2022-2023		
Service	UK Investigators	Non-UK Investigators
Cell Sorting *	\$95/hr	\$145/hr
Cell Analysis *	\$60/hr	\$92/hr
Cell analysis (self-service)**	\$40/hr	\$61/hr
AutoMACS (self-service) **	\$10/sample	\$16/sample
Consultation/Tech Support	\$25/hr	\$38/hr

* Billing for flow cytometer services is based on 15 minute intervals with a minimum charge of 30 minutes.

** Trained users only.

Immune Monitoring Service Rates for 2022-2023		
Service	UK Investigators	Non-UK Investigators
PBMC isolation	\$68	\$105
PBMC + plasma isolation	\$70	\$107
CPT tube processing	\$30	\$47
plasma/serum processing	\$24	\$37
cell counting	\$6	\$9
spleen/lymph node cell processing	\$16	\$24
FLUROSPOT assay (2 color)	\$632	\$967
Cell proliferation assay	\$58	\$89
ELISPOT assay processing	\$560	\$857
Immune phenotyping	\$41	\$62
Intracellular cytokine staining	\$48	\$73
Cytotoxicity assay (immunospot based assay)	\$103	\$157
Monocyte derived dendritic cells (monocyte differentiation)	\$147	\$225
Cytotoxicity assay (flow based)	\$42	\$65
Multiplex protein detection assay	\$136	\$208
Commercial kit based Immune assays.	\$135	\$206
lymphocyte/monocyte activation	\$100	\$153
Automacs cell separation	\$60	\$92
CTL analyzer-self service	\$100	\$153
Scientific consultation	\$65	\$99
Technical consultation	\$45	\$69

Laboratory Policies & Procedures

Policies

- All new projects for any services **must** have a **Biohazard Questionnaire** completed and sent to the facility director for approval. This is necessary to plan safe handling of samples by core personnel. The Biohazard Questionnaire form can be found on the facility webpage.
- All samples received by the Flow Cytometry Core **must** be accompanied by a **Sample Information Form** which lists the nature of the samples and any biohazards related to the samples. The sample information form can be found on the facility webpage.
- Users of the Flow Cytometry Facility must give at least **2 hours notice** of a canceled appointment (on Mondays call before 10am) in order to avoid being billed for the entire scheduled time. Billing begins from the time the appointment is scheduled to start, so please show up on time. If you are late for your appointment and run into the next person's appointment, we may be forced stop acquiring your samples and finish at a later time.
- The cell sorting flow cytometers cannot run samples that contain radioactivity or other hazardous volatile substances. Flow cytometers produce aerosols which are exposure risks for instrument operators. Consequently, analysis and/or sorting of **all potentially hazardous and/or infectious samples (including normal human cells)** must be approved by the director before arriving at the facility.

Preparatory procedures for flow cytometry and cell sorting

- Cells must be brought to the lab in Falcon 12 x 75mm polystyrene test tubes (Falcon # 2054, or Falcon #2063) for the analysis and sorting. These tubes can be purchased from UK Stores.
- Minimum sample volumes are 0.3 ml. Cells must be suspended in a medium or buffer solution containing minimal amounts of protein, i.e., 0-1% fetal calf serum or albumin.
- All samples must be filtered immediately prior to running to avoid clogging the machines. Thirty to seventy micron nylon mesh should be used. This mesh is available in Falcon brand cell strainers for 50ml conical tubes (Falcon #35 2340 - 40um and Falcon#35 2350 – 70um) and in filter top caps found on Falcon brand polystyrene 12 x 75 mm tubes (Falcon #35 2235). It is also available in sheets from Sefar America Inc. You can also add 0.5 mM EDTA or 100-200 U/ml DNase to your buffer solution to prevent clogging. We also recommend samples should be kept on ice or chilled until ready to run on the machine.
- If you request sorting, cells should be at a concentration of 10-20 million cells/ml to facilitate the sorting process. Note that these concentrations are optimal; however, lower concentrations can be run on the machines, but at slower flow rate and thus a higher cost to the investigator.

Required control tubes

1. **Unstained Sample.** This control is needed to adjust instrument sensitivity so that background fluorescence by unstained cells is reduced.
2. **Single Color stains.** These are control samples stained with just one of the fluorochromes and are used for setting compensation on the instrument so that signal spillover by other fluorochromes in the sample is reduced.

Suggested additional control tubes

3. **Isotype Controls.** Sample stained with isotype control antibodies allow identification of non-specific binding of immunoglobulin to cells in the sample. Isotype control antibodies have no specificity for target cells within a particular experiment. The isotype control antibody should have the isotype, fluorochrome and host background as the target-specific antibody to accurately assess the level of specific staining.
4. **Fluorescence Minus One (FMO).** The best way to evaluate background in samples containing multiple antibodies is to have a control that was stained with all the other fluorochromes that you will be using, except for the color detected in that channel, hence the name "Fluorescence Minus One ". This control is a must if you are trying to evaluate very dim populations in samples stained with multiple antibodies.

If you have any questions choosing the appropriate controls, do not hesitate to call Jennifer or Greg at 323-6859.

Preparatory procedures for immune monitoring services

All procedures must receive prior approval by the immune monitoring lab manager prior to bringing samples into the facility. Please call the facility to set up a meeting time to discuss and design assays for the project.