SNPRC Immunology Core Laboratory

Flow Cytometry

Policies:

1. General Facility Information

   1.1. ICL Flow Cytometry BSL-3 facility is located in Building 35, room 127. The facility is overseen and maintained by the staff of the laboratory of Dr. Luis Giavedoni in the Virology and Immunology Department of Texas Biomed; Dr. Vida L. Hodara is the facility manager. The sorter FACSAria III is situated inside of Baker Bioprotect IV biosafety cabinet designed specifically to contain a possible aerosol formed during sorting procedure. All sorting of unfixed cells in spite of being exposed or not to bacterial or viral pathogens will be managed as samples of risk following all rules of precautions. Samples labeled with radionuclides MAY NOT be sorted in this facility. The Facility Manager has the right to deny sorting of cells that are suspected to be improperly prepared, with insufficient scientific information, or having infectious agents that require higher containment than a BSL-3 level.

   1.2. The facility is available for sorting/analysis Monday through Friday from 9:00am to 5:00pm. After hours sorting may be approved on a case by case basis.

   1.3. The sorting is performed only by authorized Immunology Core Laboratory personnel. Sorting or analysis requested by TBRI/SNPRC researchers as well as by external researchers outside campus will be run after the completion of the required documentation. A risk assessment will be performed according to Biohazards and Safety Committee recommendations for all work with infectious materials. Only approved research projects may be conducted in this BSL-3 lab.

   1.4. Sort request forms must be given to the Flow Cytometry Facility Manager a minimum of 72-hours prior to the requested sorting date.

   1.5. The Immunology Core Sorting facility must be notified of any cancellations by 5 PM on the day prior to the sorting date.

2. Specimen Requirements:

   2.1. Living cells samples must be suspended in cell culture medium or buffer, and filtered through a 100 µm mesh (i.e. Falcon 2235 tubes with integrated filter cap, CellTrics sterile filter caps from Partec; SpectraMesh nylon filters, Miltenyi Biotech MACS pre-Separation Filter Cat# 130-041-407) to remove clumps, and placed on ice prior to arrival to the Facility. In the case of viruses or microparticles, or plasma samples, all buffers and media used to wash or dilute them should be previously filtered with 0.22 µm pore size filters or an appropriate filter to eliminate precipitates or unwanted clumps.

   2.2. Users are responsible for contacting the facility manager to discuss proper sample preparation prior to sorting. This will include single cell suspensions not to exceed 2.0×10^7 /ml, and nozzle size considerations. Generally, nozzle opening at least four times larger than the cell diameter is recommended. Nozzles are available in 70 µm and 100 µm diameters.

   2.3. Samples must be in an appropriate tube for sorting (e.g. 12 x 75 mm snap-cap tube, 15 ml polypropylene conical tube).

2.4. A separate individual set of stained (control) cells for each flurochrome in addition to an unstained (preferable isotype immunoglobulin stained) sample is required for setting up the experiment. If more than 4 colors are used, a set of cells or antibody-capture beads stained as single color controls with each antibody used in the experiment will be provide if possible to perform the compensation set-up. These samples should be at or near $1x10^6$ cells/ml and not less than 400 µl.

2.5. For all fixed specimens, appropriate and reliable methods must be used to inactivate potentially biohazardous agents (i.e. freshly prepared 2% formalin solution for 30 minutes).

3. Materials Required:

3.1. Users must supply enough appropriate collection tubes (12 x 75 mm or 15 ml conical) for each sample to be sorted. If sorting into plates rather than tubes, the corresponding plates should be provided for collection plus one more plate for aligning the sort stream.

3.2. If cells are required to be sorted into any special medium or buffer, that should be provided.

3.3. If sorting rare events in the sample that will take a long time to sort, 20-40 liters of sorting buffer (Sterile filtered one percent FBS, Ca^{++} and Mg^{++} free 1X PBS, 1mM EDTA, 25 mM HEPES PH 7.0) to be used as sheath fluid.

4. Entering to the BSL-3 laboratory:

4.1. Before entering the BSL-3 lab the user must sign in a log.

4.2. Only individuals who are trained may enter the laboratory. When setting of the sorter requires to have the owner of the samples present, she/he will be allowed to enter in the lab only if escorted by the Sorter Operator and wearing the proper PPE, and to leave the laboratory immediately before starting the sorting procedure.

4.3. Before entering to the BSL-3 laboratory the user has to obtain the PPE required (lab gown, shoe covers, gloves, goggles or safety glasses). When sorting unfixed samples the operator will also wear a PAPR. All PPE are located in the ante-room.

4.4. Wear a second pair of gloves that you can change if touching a spill inside the lab keeping the first pair on. In this way it will not be any skin contact with materials inside the laboratory.

4.5. Put on all PPE at the entrance of the laboratory, once the red tape on the floor has been passed the personnel cannot step back into the clean area with the shoe covers or contaminated PPE.

4.6. Ensure the self-closing laboratory door is properly closed behind you.