Flow Cytometry Core, KRCBS

Flow Cytometry Core Facility – External Investigator Sample Registration

- A Sample Registration must be presented for each sample type in order to inform the operator of the biohazardous risk that the samples represent.
- Each new experiment using a new sample type, cell line, or vector which has not been registered previously must be accompanied by a new Sample Registration form.

PI Name: **PI Email:**

Lab Member Name: **Lab Member Email:**

Sample Information:

a. **Cell type (i.e.: immortalized cells, primary cells, tissue digests, blood sample):**

b. **Species of origin (i.e. human, murine, etc.):**

Intended Markers and the conjugated Fluorophore(s)/Dyes: (e.g. CD45-APC-Cy7, CFSE):

Staining (extracellular, intracellular, both):

◯ Extracellular ◯ Intracellular ◯ Both

List of chemical treatments (i.e. LPS):

List of infectious agents (i.e. lentivirus):

List of transfected vectors/genes (i.e. pLEN Ras-GFP):

What viability stain will you be using?

Analyzers available at the facility: BD Fortessa X-20, Beckman CytoFlex LX, Sony SP6800, (please indicate if you have a preference):

Indicate if you require Flowjo analysis post acquisition? (Y/N):

◯ YES ◯ NO

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Please note: All RG2 biohazardous agents run on analyzers **should be fixed.**

- RG2 agents: human primary cells or cell lines from human and animals containing RG2 agents (i.e. retroviral vectors, etc.)
- the standard fixative used is **1-2% paraformaldehyde** for at least 15-30 minutes on ice (agent dependent)

If required the Flow Cytometry Core staff (monika.lodyga@unityhealth.to) and Research Biosafety Officer (neha.chauchan@unityhealth.to) can be contacted to help with the completion of this form

- Note: all users are required to adhere to the Policies of the LKSKI Flow Cytometry Core, specifically the cleaning and maintenance requirements for the instruments.

I_____, declare that the above information is accurate and that no undeclared

(Name)

Biological safety risks exist to the operator or other users of the Flow Cytometry Core facility.

Date: Signature:

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Appendix: Sample Preparation Requirements:

What buffer to use?

Basic flow buffer (non-adherent cell types)

- 1x PBS or HBSS (Calcium/Magnesium free)
- 1-2% FBS
- Filter sterilize using a 0.22 µM filter
- Store at 4 degrees

Flow buffer (adherent cells or tissue digests)

- 1x PBS or HBSS (Calcium/Magnesium free)
- 1 % BSA
- 1 mM EDTA (will keep more sticky cells from re-associating)
- Filter sterilize using a 0.22 µM filter
- Store at 4 degrees

What controls to bring?

- **Unstained control**

- **Single stain controls**
  - Will reveal the level of spectral overlap between different fluorophores and allow you to remove or compensate for this overlap.
  - Stain your fluorochrome-conjugated antibody on the experimental cell type or on antibody capture beads.
  - the single stained control must contain a **positive** and **negative** population AND the autofluorescence of the positive and negative populations must be equivalent (same particle types)
  - **If population is rare or requires induction** (use compensation beads) i.e.:
    - UltraComp eBeads™ Plus Compensation Beads (ThermoFisher, Cat# 01-3333-42)
    - OneComp eBeads (ThermoFisher, Cat# 01-1111-41)
    - AbC™ Total Antibody Compensation Bead Kit (ThemoFisher, Cat# A10513
      ***Always double check that the beads are compatible with your antibodies.

- **Live/dead control (viability dye):** allocate an aliquot of cells of interest, heat treat at 65 degrees for 4 min, immediately place on ice for 1 min. Following the treatment, the heat-killed cells can be combined 1:1 with live cells and then stained with your viability dye.

- ***FMOs** (Fluorescence minus one) controls - gating controls for your experiment:
  - Use same cell type as your sample, stained with all the fluorochromes minus one fluorochrome (see table below).

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FMO Control Setup - Example:

Marker	Fluorophore	APC FMO	AF700 FMO	PE FMO	PE-Cy7 FMO
CD45	**APC**	-	+	+	+
CD4	**AF700**	+	-	+	+
CD25	**PE**	+	+	-	+
CD8	**PE-Cy7**	+	+	+	-
	DAPI (viability)	+	+	+	+

Important: Clumps and debris can clog the instrument fluidics and either distort the measurements or obstruct them completely.

- **Samples should be passed through a 35-50µm nylon mesh just prior to acquisition. i.e.:**
  - 5 mL polystyrene, round-bottom tube with 35 µm nylon mesh cell strainer snap cap (Falcon, #352235)
  - pluriStrainer Mini 40 µm (just filter cap) (puriSelect, #43-10040-40)
- Please bring your samples in **5ml Falcon polystyrene tubes only** (Falcon, #352008)

Other items that you may need for acquisition of your samples:

- Secondary container with lid (keep samples on ice and protect from light)
- P1000 pipette and tips
- Extra flow buffer (5-10 ml)
- Extra 5ml tubes and cell strainers

Please return the completed form to monika.lodyga@unityhealth.to