Flow Cytometry Core, KRCBS.

Flow Cytometry Training Information Form

Date:

Name:

Lab:

Background Flow Cytometry Experience (if yes, what instrument?):

Research Aim/Question:

Sample: (e.g. immortalized cells, primary cells, tissue digests, blood sample):

Markers/Fluorophores/Dyes (e.g. CD45-FITC):

Viability dye:

Staining (extracellular, intracellular, both):

What samples/controls will you be bringing to the training?

Availability for training (please provide a few dates, with a 2hr window):

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

Indicate if analysis software training is required (Flowjo)? (Y/N):

◯ YES ◯ NO

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)

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 samples 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.
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- **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 for multicolor experiments = 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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- **All stained**: minimum of two fluorchoromes + viability dye.

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.:**
  - Falcon® Round-Bottom Tubes with Cell Strainer Cap, 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)
- **Note: Costs incurred due to misuse of instrument (clogging, damage) will be charged to the lab**

Other items that you may need during the hands on training:

- ice 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 bring your samples in **5ml Falcon polystyrene tubes only** (Falcon, #352008) , other tubes will not form a proper vacuum seal on the sample injection port (relevant to: BD Fortessa X-20, Sony SP6800)

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