



**Flow Cytometry Core, KRCBS**

### Flow Cytometry Core Facility – Sorting 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.

**User Name:**

**User Email address:**

**PI Name (Institute):**

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**Sample type and details\*:**

**Cell Type** (species, primary or immortalized):

**Name of Cells** (i.e. HeLa):

**Intended Markers and the conjugated Fluorophore(s)** (i.e. CD45-FITC):

**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:**

**Volume of collection tubes:** 5ml or 15ml (polypropylene):

5 ml

15 ml

**Population(s) of interest to be sorted:** ie. (CD4+/CD25+) and (CD4/CD25-)

**Number of samples to be sorted:**

**The user declares that the above information is accurate and that no undeclared biological safety risks exist to the operator or other users of the Flow Cytometry Core facility.**

## Sorting Sample Preparation Requirements:

### Basic sorting buffer:

- 1x PBS or HBSS (Calcium/Magnesium free – prevents clump formation)
- 0.5 % BSA
- 1 mM EDTA (will keep more sticky cells from re-associating)
- 25 mM HEPES pH 7.0
- Filter sterilize using a 0.2 µM filter
- Store at 4 degrees

**Note:** Use 0.5% BSA if possible, instead of the 1-2% as per the standard used for analyzer acquisitions, presence of more serum protein causes more clogging issues

- **Cell concentrations (cell size type dependent)**
  - 40 million/ml -70um nozzle
  - 20 million/ml -85um nozzle
  - 10 million/ml -100um nozzle
- **Filter sample just prior to the sort**
  - Pass the samples through nylon mesh with a pore size of 35-40 µM to eliminate large aggregates 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)
- **Bring your samples in a 5ml Falcon polystyrene tubes** i.e.:(Falcon, #352008)
- **Viability dye is a mandatory requirement** (we can select one once we know the details of your panel)
- **Collection tubes:**
  - 5ml or 15 ml, sterile **polypropylene tubes**
- **Collection medium:** it is common to collect sorted cells into medium containing 50% FBS **or just FBS**. As the sorted cells are collected the FBS is diluted to approximately 50% which helps retain cell viability
  - 2 – 3 mL for 15 mL tubes
  - 750 µL - 1 mL for 5 mL tubes
- **Controls**
  - Unstained cells
  - Live/dead control
  - Compensation controls if required
  - Fluorescence minus one (FMOs) - for multicolor experiments

Please return the completed form to [monika.lodyga@unityhealth.to](mailto:monika.lodyga@unityhealth.to)

**Cytometer Fluorochrome Compatibility Reference –BD Aria III FACS**

| <b>Laser<br/>(Excitation)</b> | <b>Axis<br/>Label in<br/>DIVA</b> | <b>Bandpass<br/>Filter</b> | <b>Common<br/>Fluorophores</b>   | <b>Viability<br/>Dyes<br/>(*Fixable)</b>                                | <b>Nuclear<br/>Dyes</b> | <b>Cell Cycle/<br/>Proliferation</b>            |
|-------------------------------|-----------------------------------|----------------------------|--|---|-------------------------|---|
| 407 nm                        | BV421                             | 450/40                     | BV421,<br>Pacific Blue,<br>VioBlue,<br>Alexa405,<br>Calcien Blue       | FVS450*,<br>SYTOX<br>Blue,<br>Zombie<br>Violet*                         | DAPI                    | VPD450,<br>CellTrace Violet,<br>DyeCycle Violet |
| 407 nm                        | BV510                             | 510/50                     | BV510,<br>Pacific Green,<br>AmCyan                                     | FVS510*,<br>Zombie<br>Aqua*   |                         |   |
| 407 nm                        | BV605                             | 610/20                     | BV605,<br>Pacific<br>Orange<br>SB600                                   | Zombie<br>Yellow*   |                         |   |
| 407 nm                        | BV650                             | 660/20                     | BV650  |   |                         |   |
| 407 nm                        | BV711                             | 710/50                     | BV711  |   |                         |   |
| 488 nm                        | FITC                              | 530/30                     | FITC,<br>Alexa488,<br>GFP, Calcien,<br>DHR, BB515,<br>DCF, DiO<br>dyes | FVS520*,<br>Live/Dead<br>Green*,<br>SYTOX<br>Green,<br>Zombie<br>Green* |                         | CFSE, Dye Cycle<br>Green                        |
| 488 nm                        | PerCP-<br>Cy5.5                   | 695/40                     | PECy5.5,<br>PerCP Cy5.5,<br>PerCP                                      | 7AAD  | LDS751                  |   |
| 561 nm                        | PE                                | 582/15                     | PE   |   |                         |   |
| 561 nm                        | PE-<br>TexasRed                   | 616/23                     | PE-<br>TexasRed,<br>PE-CFS594<br>mCherry                               | PI, 7AAD  |                         |   |
| 561 nm                        | PE-Cy5                            | 660/20                     | PE-Cy5   | PI, 7AAD  |                         |   |
| 561 nm                        | PE-Cy5.5                          | 720/40                     | PE-Cy5.5   |   |                         |   |
| 561 nm                        | PE-Cy7                            | 780/60                     | PE-Cy7   |   | LDS751                  |   |
| 633 nm                        | APC                               | 660/20                     | APC,<br>Alexa647,<br>Alexa660,<br>Alexa 680,<br>DiD, SB645,<br>BV650   | Live/Dead<br>Far Red*,<br>FVS660*,<br>SYTOX<br>red                      | Draq5                   | Dye Cycle Ruby                                  |
| 633 nm                        | AF700                             | 730/45                     | AF680,<br>AF700,<br>Qdot705<br>BV711                                   |   |                         |   |
| 633 nm                        | APC-Cy7                           | 780/60                     | APC-Cy7,<br>APCH7<br>SB780,<br>BV786                                   | Zombie<br>NIR*  |                         |   |