LS&E
Infrastructure Center

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Sample preparation for Analysis @ LSE:

☐ Samples preparation:

- Prepare a single-cell suspension.
- For whole blood use red blood lysis buffer (or if you have RBC contamination).
- Count cells and adjust to a concentration of 1-2X10^6 cells/ml.
- Use Fc receptor blocking to reduce non-specific binding.
- For intracellular markers (cytokines, transcription factors, etc), fix and permeabilize cells before staining.
- Add viability dye like 7AAD and PI to exclude dead cells.

☐ Samples needed:

- Unlabelled cells (unstained).
- Single-stained (for each fluorophore).
- Mix-stained sample (usually your sample).
- Biological control, depending on the experiment.
- FMOs (Fluorescence Minos One) - for high parameter panels (mixed stained sample without one fluorophore, needed for each fluorophore).
- Calibration beads - consult with us if needed.

☐ **Volume:** For all analyzers, the minimum workable volume is 300ul for tubes,

and 100-150ul for 96-well plate

☐ Maintaining viability

Suspend cells in the appropriate buffer to prevent cell clumps: For non-adherent cells, a basic suspension buffer of $PBS^{-/-}$ (no calcium, no magnesium) supplemented with **1–2% FBS or BSA** works well. You may need to add **1mM EDTA/EGTA (0.5-2mM)**, especially if you have adherent/sticky cells, as it helps chelate divalent cations that are often required for the formation of cell aggregates. Additionally, if you have a high percentage of dead cells, adding **DNase I** (100 µg/ml or 10 units/ml) can reduce clumping caused by free DNA (DNase I requires 1-5mM Mg^{2+} {optimal 5mM} as a cofactor).

DNase I protocol: concentration varies 10-100 µg/ml (adjust based on % dead cells)

- Magnesium: Add 5mM $MgCl_2$ to buffer.
- Incubation: 15-30 min at room temperature.
- Maintenance dose: 25-50 µg/ml during sort to prevent re-clumping.
- Important: Actin released from dead cells irreversibly inhibits DNase I.

It's important to do the DNase I treatment first and only after the incubation ends to add the EDTA (since both chelate Ca^{2+}/Mg^{2+})

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☐ **Filtration:** Filter the cells through a nylon mesh. The mesh size depends on the **size of your cells**:

40µm mesh or 70µm mesh (BD Falcon™ cell strainers ref. 352340, 352350, respectively).

* **Take into consideration your cell size; do not use a mesh size similar to your cell size**!

☐ **Temperature:**

If you bring your cell in at 4°C, it is advisable to keep the analyzer at the same temperature, since thermal shock can significantly reduce viability. Only available in plate mode- on Cytoflex lx and Cytek Aurora.

For any other questions, please feel free to consult with us:

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