LS&E Infrastructure Center

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Sample preparation for sorting @ LSE:

☐ Pre-sort preparation:

Cell viability should be checked using trypan blue or a viability dye.

Optimal viability should be ≥95%.

Minimum starting viability: 70% (below this, consider optimization).

Viability below <70%, high dead cellular percentage, may compromise DNase I effectiveness and could result in poor recovery.

For sensitive cells that are prone to sticking, pre-coat the collection tubes with 1-2% BSA/FBS in PBS-/-(without Ca^{2+}/Mg^{2+}) for at least 10 seconds while agitation.

☐ Most common viability Dye:

DNA-Binding Dyes (Live cells only – impermeable membrane):

- DAPI: 0.1-1.0 µg/ml (optimal 0.2 µg/ml); add immediately before acquisition; keep on ice
- PI: 1 µg per 100 µl sample; 5-15 min incubation; no wash required

Fixable Viability Dyes (Live or fixed):

- Zombie Dyes: 1:100 to 1:1000 dilution for 1-10 million cells (important stain in PBS without protein {FBS/BSA reacts with amine-reactive dyes}), incubation: 10-15 min at RT or 4°C, protected from light.

☐ Samples needed:

Unlabelled cells (unstained).

Single-stained (for each fluorophore).

Mix stained sample (usually what you sort).

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.

☐ Cell concentrations:

For a standard 100 µl nozzle size -

- For BD ARIA 5-6X10^6 Cells/ml
- For Thermo Bigfoot 3-5X10^6 Cells/ml

☐ **Volume:** For both sorters, the minimum workable volume is 500ul

☐ **Nozzle size and pressure:**

BD - Aria IIIU

Recommended Cell Size (µm)	Nozzle Size (µm)	Typical Pressure (psi)
<15 µm	70 µm	70 psi
15-17 µm	85 µm	45 psi
17-22 µm	100 µm	20 psi
>22 µm	130 µm	11 psi

Thermo - Bigfoot

Recommended Cell Size (µm)	Nozzle Size (µm)	Typical Pressure (psi)
≤ 14	70	60 psi
≤ 20	100	30 psi
≤ 24	120	20 psi
≤ 30	150	8.75 psi
≤ 40–50+ (or rare, large cells)	200	6 psi

Key principle: Nozzle should be ≥5× the cell diameter

☐ **Sorting tubes:**

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). It's strongly recommended to add **25mM HEPES (pH 7.0)** to your sorting buffer (HEPES provides superior pH buffering capacity throughout the sort process. During high-pressure sorting, CO_2-buffered media lose pH stability when exposed to atmospheric conditions, causing the buffer to become basic.

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+})

☐ Collection tubes:

- Collect up to four/six (Aria/Bigfoot) different populations into 1.5ml and 5ml tubes.
- Up to two/four (Aria/Bigfoot) populations for 15ml tubes and for 2X 50ml tubes.
- Sort into all plate types. 6-well,12-well, 24-well,36-well, 96-well, 384-well, PCR plate, etc.

*It's very important to sort into **polypropylene tubes** (and not polystyrene, since it accumulates static electricity)

Collection tubes should contain a collection buffer. The most recommended collection buffer is your **cell culture medium with 10% FBS** or some other serum. Collection tubes should be about 1/3 full of collection media.

Addition of an **antibiotic** (Pen-Strep, Gentamicin, Ampicillin, Erythromycin, etc) to the collection tube is required. Please make sure to add a higher concentration of antibiotic since the collection tube will be diluted from the sheath buffer.

Dilution factor: 1 million sorted cells adds ~1.5ml (70µm nozzle) to 3.5ml (100µm nozzle) of sheath fluid.

☐ *Bring extra Medium/PBSX1 for further dilution of your sample if needed

☐ *It's always recommended to include live/dead dye: DAPI, PI, Hoechst, zombie, etc.

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

40µm mesh for 70µm nozzle (BD Falcon™ cell strainers ref. 352340)

70µm mesh for 100µm nozzle (BD Falcon™ cell strainers ref. 352350).

The best option is the Falcon® 5 mL Round Bottom Polystyrene Test Tube that comes with an in-built 35um filter (Corning ref. 352235).

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

☐ Filtrations:

2 Filtrations are needed:

1. After washing the cells from staining
2. Immediately before entering the cells into the sorter

☐ Sorting Conditions:

If you bring your cell in at 4°C, it is advisable to keep the collection tubes at the same temperature, since thermal shock can significantly reduce viability. However, we can also warm the sorter to 37°C in accordance with your cell's needs, maintaining consistent temperature throughout the workflow.

Please inform us of what sorting conditions you'll require.

☐ **Post sorting:** It's recommended to centrifuge the cells and resuspend them in their appropriate growth media (with antibiotics)

Cell counting after sort: Recommend post-sort assessment of viability and yield to calculate recovery.

Recovery rate: (sorted cells counted / theoretical cells) × 100, typical recovery: 75-95%,

with viability >90%

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

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