Sample Preparation Guidelines for Cell Sorting

UWCCC Flow Cytometry Laboratory
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Cell viability, autofluorescence, and cell aggregation may all affect the overall quality of live cell sorting experiments. Superior cell preparation is crucial and will result in better sort purity, yield, and post-sort cellular function and viability. Please consider the following guidelines for cell preparations intended for cell sorting.

Buffer Suggestions

Use Ca^{++}/Mg^{++}-free buffers. PBS without Ca/Mg is advised. This helps to reduce cell aggregation.

Include 0.1-1% BSA or dialyzed FBS at 1-5%. Use a minimal amount of BSA to decrease autofluorescence and to increase population resolution. Avoid non-dialyzed FBS, as it facilitates cell-cell adhesion by replacing Ca and Mg.

Add EDTA at 2-5mM to help prevent cell adhesion.

High pressure during sorting compromises buffer capacity. Add 10-25mM HEPES to improve pH stability.

To samples with reduced cell viability, omit EDTA and add 25-50 ug/mL DNAseI with 5mM $MgCl_2$. This digests free DNA released by dead cells.

Single Cell Suspension

Filter immediately before sorting. Filters are available in the Flow Lab.

When processing tissue samples, pass cells through a 25-gauge needle.

Avoid keeping cells at unnecessarily high concentration. Keep the cell suspension at 1-10 million/mL during processing, depending on cell type.

Dead Cell Discrimination

We strongly suggest using a dead cell exclusion dye with any cell sorting experiment. This will greatly reduce autofluorescence and lower non-specific baselines, which will ultimately result in increased population resolution. There is a good selection of dyes for live samples, which will not affect cell physiology for post-sort functional assays.

Physical Manipulations

Centrifugation
Use minimal speed to sediment cells. A good starting point for most preparations is 300xg for 10 minutes.

Vortexing
Avoid vigorous vortexing.

Pelleting
Do not generate a dry pellet at any time during processing.

Air Bubbles
Avoid introducing air bubbles. Surface tension forces can kill cells.

Temperature
Keep samples on ice, unless otherwise required by a specific protocol. Slowing intracellular metabolism helps cells survive longer outside the incubator.

Filter Information

Disposable option:
5mL tubes with 35um mesh caps
Fisher catalog number 0877123

Reusable option:
CellTrics 0400422316 plastic filters
Elko 03-41/31 41um nylon mesh
Wash plastic with Alconox, rinse with water, insert new squares of nylon mesh cut from large rolls

Compensation Controls

If using cells for single color compensation controls, bring unstained cells as the negative control for compensation.

If using beads for compensation, include unstained beads for compensation (as an internal population or a separate tube) and bring unstained cells for an additional technical control.

Gating Controls

Provide proper gating controls. This is essential even for single-color sorts.

FMOs are highly recommended for multi-color assays.

Additional biological controls may be necessary for proper gating, including:

- Negative Control
- Positive Control
- Mock Transfected
- Treated
- Untreated

Sample Delivery

Samples must be contained in leak-proof tubes, and transported in a leak-proof container and a sealed cooler.

Cells are typically sorted at approximately 10 million per mL, depending on cell type.

If there are fewer than 5 million cells in a sample, resuspend in 300-500uL.

Bring extra sample buffer (5-15mL), FBS, collection buffer, and collection tubes as backups.

Provide proper collection tubes for your application (e.g. sterile, RNAse-free). The Flow Lab does not provide RNAse-free collection tubes.

Bring extra ice or dry ice for long sorts, if necessary.

Biosafety Pre-Approval

Prior to any sort requests, all sorting experiments must be described in the Biological Safety Protocol and approved by the OBS. The protocol number must be entered in the appointment form when scheduling a sort.

Collection

Recovery

Small numbers of cells should be collected into small tubes for best recovery.

Consider collecting directly into plates or lysis buffer when cell numbers are low.

Viability

Coat tubes with protein to improve viability. Avoid empty collection tubes!

Many cells will not tolerate 100% FBS.

Standard Formats

Tubes

- Microfuge tubes (1-4 populations)
- 12x75mm 5mL tubes (1-4 populations)
- 15mL tubes (1-2 populations)

Multi-well plates (1 population)

- 6, 12, 24, 48, 96, or 384 wells

Custom Formats

When sorting onto a custom device (not listed above), please provide a template at least 30 minutes prior to your appointment. This allows the operator to aim the sort streams for the custom device.