Flow Post-its
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Sample Preparation Tips for Cell Sorting

Feb 2020

Cell Viability, autofluorescence and cell aggregation may all affect the quality of cell sorting experiments. Good sample preparation is crucial and will result in better sort purity, yield and post-sort cell function and viability.

1. Buffer Suggestions

- Use Ca^{++}/Mg^{++} free buffers → reduces cell aggregation
- Use BSA (0.1 – 1%) or dialyzed FBS (1 – 5%)
  - Minimal amount of BSA decreases autofluorescence, increases population resolution, and improves flow rate in instrument
  - Avoid non-dialyzed FBS as it facilitates cell-cell adhesion by replacing Ca and Mg
- Add EDTA (2 – 5 mM) → helps prevent cell adhesion
- Add 10 – 25 mM of HEPES to improve pH stability
- Add DNAse I (25 – 50 ug/mL) and 5 mM of MgCl2 → digests free DNA released by dead cells.

2. Single Cell Suspension

- Filter samples immediately before sorting
- Avoid keeping cells at unnecessarily high concentration. Keep cell suspension at 1 – 10 million/mL during processing.
- Always check cells under the microscope as you are preparing them to ensure they are in a single cell suspension.

3. Dead Cell Discrimination

- Strongly recommended to use a Dead Cell Exclusion Dye with any cell sorting experiment. It will greatly reduce autofluorescence and lower non-specific baselines, which will result in increased population resolution.

To keep in mind:

- Use minimal speed to centrifuge the cells.
  A good starting point is 300 g for 10 minutes.
- Avoid vigorous vortexing.
- Do not generate a dry pellet at any time during processing.
- Avoid introducing air bubbles, since surface tension forces can kill cells.
- Keep cells on ice, unless otherwise required by a specific protocol.