Considerations for Cell Sorting

BD FACSAria

The BD FACSAria is a fluorescent activated cell sorter capable of physically isolating cells to purify them for a variety of downstream applications. A successful sort is dependent on a variety of factors, including sample preparation, inclusion of controls, instrument setup and sample tube or plate preparation.

Sample Preparation

All samples must be in a single cell suspension and filtered with the appropriate size mesh strainer prior to acquisition. Failure to do so can cause instrument clogs, leading to increased biohazard potential and instrument down time.

- **Dissociation Buffer** A variety of cell dissociation buffers are available (Trypsin, Accutase, TryPLE. Cell Dissociation Buffer, etc). Optimization may be required for different cell lines or tissue types.
  Note: If staining for cell surface markers, ensure the buffer used does not alter epitopes of interest.

- **Single Cell Suspension** Before you stop the reaction for dissociation, check your cells under the microscope to ensure that a majority of the cells are in a single cell suspension. Stopping the reaction too early can lead to large aggregates and poor sorting results.

- **Resuspension Buffer** PBS alone may not be sufficient for a resuspension buffer. We suggest to use CAMg^{++} free buffer with 0.5% BSA or 2%FBS & 2mM EDTA to reduce cation-dependent cellular re-adhesion.

- **Low Viability** If high cell death is seen, the addition of 25-50μg/mL DNAse I will prevent re-adhesion of cells due to extracellular DNA.
  Note: EDTA cannot be used in conjunction with DNAse.

- **Filtration** ALL samples must be filtered immediately prior to running on a cell sorter. Keep in mind that filtration does not help with generating a single cells suspension, but only removes large aggregates of cells.

- **Tube Considerations** If possible, samples should be brought in 5mL polypropylene tubes. All samples should be brought in *solid cap* tubes. Filter cap tubes are open to the environment and can lead to contamination of your samples.

- **Temperature** Keep your cells at the appropriate temperature for optimal health. Not all cells prefer 4C. If you prefer to keep your sort samples at 4C or room temperature, alert the sort operator at the time of your sort to your preferences.

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Controls

Appropriate technical and experimental controls should be run at the time of each experiment in order to have confidence in the results.

- **Unstained** A fully unstained sample (without viability dye or any fluorescent protein) is necessary to see the autofluorescence of your cells. Parental unstained will be necessary for any cells that are expressing fluorescent proteins.
- **Viability Alone** Parental cells that only have viability dye added to the sample. This control is necessary to determine the appropriate gating and autofluorescence of live cells, excluding dead cell populations.
- **Single Color** Whenever an experiment is run with more than one fluorochrome, single color controls must be acquired in order to calculate compensation to correct for fluorescent spillover. Single color controls need to be the same fluorochrome that is being used in the experiment and should be as bright or brighter than experimental samples may be beads or cells and should only have one fluorochrome.
  Note: Viability dye should not be added to single color controls
- **FMO Controls** *F*luorescence *M*inus *O*ne controls are experimental samples that contain all antibody fluor conjugates with the exception of one. These controls are used to help determine appropriate gating on your samples when spreading is seen.
- **Secondary Antibody Alone** If you are using an unlabeled primary antibody in combination with a fluorochrome conjugated secondary antibody, a control only stained with the secondary antibody is strongly advised. This control will determine if there is non specific binding of your secondary antibody to your sample.
- **Negative Control** A sample that is known to be negative for your marker of interest stained with the experimental antibody to ensure there is no cross reactivity or false positive results.
- **Positive Control** A sample that is known to be positive for your marker of interest stained with the experimental antibody to ensure it is performing as expected.
- **Unstimulated Control** An unstimulated control sample stained with all of your markers of interest to set a baseline for expression levels in your panel.

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Instrument Setup & Suggested Concentrations

The BD FACSAria cell sorter can run at a variety of pressures utilizing different nozzle sizes. As a standard practice, the nozzle being used for a cell sorting experiment should be at least 5 times the diameter of the dissociated cells being sorted. Extra consideration should be taken when sorting sensitive cells (activated T cells, neurons, etc) to reduce pressure and/or increase nozzle size.

Nozzle Orifice	70µm	85µm	100µm
Cell Size	≤14 µm	≤17 µm	≤20 µm
Concentration*	20 x 10^6 cells/mL	15 x 10^6 cells/mL	10 x 10^6 cells/mL
Pressure	70 psi	40psi	20psi
Event Rate	~18,000 evts/sec	~8,000 evts/sec	~6,000 evts/sec
# Events/hr	~65 x 10^6	~28 x 10^6	~21 x 10^6

**Note: It is suggested to bring extra buffer. If the samples are too concentrated, extra buffer can be added at the sorter to optimize the event rate.*

Specialized Applications

Cell Cycle: When carrying out cell cycle experiments, it is preferred to run at a slower flow rate to reduce spreading of the data. Concentrate your sample according to an optimized protocol and run at lower flow rate for reduced CVs.

Single Cell Sorting: Many cell types are under stress when they are sorted individually into a well. To mitigate for this, it is suggested to sort at a lower pressure (100µm nozzle @ 20psi) to lessen the stress on the cells during sorting. The use of conditioned media as a collection buffer should be considered if possible.

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Collection Tube/Plate Considerations

The BD FACSAria cell sorter can sort into 1.5mL, 2mL, 5 mL and 15mL tubes or 6, 12, 24, 48, 96 & 384 well plates. To help ensure a successful cell sorting experiment, follow the suggestions below.

Use Polypropylene Due to the charging of the droplets, polystyrene is preferred over polypropylene for collection tubes to prevent adhesion of the charged droplets to the plastic.

Pre-coat Tubes Empty (dry) collection tubes or plates should never be used. As the droplets are being sorted, they will desiccate quickly or potentially bounce out of the collection tube/plate. To prevent this, pre coating the tubes is suggested.

- Tubes can be filled with whole serum, PBS with 20%FBS or PBS with 5%BSA. Leave tubes at room temperature for a minimum of 2 hours, or at 4C overnight. Prior to the sort, remove excess serum or empty completely and replace with collection media

Collection Media

- Use the appropriate collection media to ensure your cells are as healthy as possible after the sort. Consider enriching the media with serum if it benefits your cells of interest.
- Add antibiotics/antimycotics to the collection media to prevent any contamination of your samples. While the sorters are enclosed in BSCs, sterility is not guaranteed.
- Consider the use of lysis buffer for downstream genomics applications.
  Note: Certain buffers, such as Trizol LS, require specific dilutions of buffer to cell suspension to work properly. Consult with flow core staff in advance of your sort to discuss optimal volumes.

Suggested Reading

Arnold, L.W. and Lannigan, J. (2010), Practical Issues in High-Speed Cell Sorting. Current Protocols in Cytometry, 51: 1.24.1-1.24.30. https://doi.org/10.1002/0471142956.cy0124s51