Cell Sorting Guidelines

Instrument

The MoFlo (Dako Colorado, Inc.) is configured with:

1. iCyt 200S Laser System laser, tuned to 488 nm excitation with variable power;
2. A solid state red laser tuned to 635 nm excitation.
3. forward and side scatter detectors plus 7 color analysis PMTs;
4. 1, 2 or 4 -way cell sorting at a rate of up to 70,000 cells per second (cps)
5. A CyClone, a robotic arm to directly deposit sorted cells onto microscope slides or dispense sorted cells into the wells of microplates;
6. A Smart Sampler loading platform that accepts a wide variety of the most commonly used tube formats; including 0.5 ml microcentrifuge, 1.5 ml microcentrifuge, 5 ml round bottom, 15 ml conical and 50 ml conical;
7. A cytoshield aerosol filtration system to protect the operator from hazardous aerosols
8. A circulating water bath which can be adjusted to any temperature for the sorting sample and recovery tubes.

Scheduling

1. Make an appointment with Allison Church Bird at least two days in advance. Remember my motto BOOK EARLY BOOK OFTEN!
2. Please submit the Sample Submission form in advance so that the instrument can be configured for your sort.
3. If you need to cancel your sorting appointment, please do so as early as possible.
4. If you are running late for your sort appointment, please notify ALI (4-2711). Please keep in mind that charges for laser time begin when the laser is first fired up.

What to Bring

1. Your cells (Maximum concentration for sorting is 10 million cells per ml).
2. Extra media/buffer to dilute your cells if over concentrated.
3. FBS/media (plan on 2-4 ml per 1 x 106 sorted cells) in your sorting tubes; for 4 ways sort these are 5 ml round bottom polypropylene tubes; for 1 or 2 way 15 ml or 50 ml tubes can be used
4. A sample of unstained cells
5. For multicolor analyses, compensation controls for each color used

Sorting Speed

1. cell type
2. cell concentration
3. sort mode [purity or enrichment or single cell]
4. frequency of the target cells
5. tip size
6. contaminating debris

For example, when sorting at a sample rate of 10,000 cells per second, 36 x 10^6 million cells can be processed per hour. The cell concentration should be no higher than 10 million cells per ml. Cell preparations with high amounts of non-cellular debris can interfere with the sort rate and efficiency.

Cells to Be Sorted

Count your cells immediately prior to sorting as this will allow us to estimate the efficiency of yield and to trace problems associated with a low yield. From experience, we have found that the staining process results in considerable cell loss, usually through centrifugation. We highly recommend that you **prepare twice the number of cells needed for a sort**. Immediately prior to sorting, cells will be ***filtered*** through nylon mesh. See filtration protocol..

Cell Media

Cells should be resuspended in a low protein buffer, such as Ca++/Mg++free PBS (or phenol red-free HBSS). These are the supplements that are highly recommended to reduce cell aggregation:

1. Ca/Mg free buffer to prevent macrophages/monocytes from sticking to tubing
2. 5 mM EDTA to prevent macrophages/monocytes from sticking to tubing; Some activated cells become clumpy and the chelators (EDTA) help reduce cation-dependent cell to cell adhesion
3. 0.5-2% BSA [not FBS]
4. 1% heat-inactivated FBS [dialyzed against Ca/Mgfree PBS]; <2% FBS
5. DNase at 350-500 Kunits/ml; 10U/mL DNAase II to remove DNA-induced clumpiness
6. Other agents can also be useful for separation of cells from solid tumors, ask the operator for advice.

Number of Cells Required

To help maximize cell recovery, we need to know:

1. how many cells are present in your unsorted population
2. the estimated frequency of the target population
3. the number of cells that you require after the sort
4. the desired result; high cell yield or high purity

Instrument Sterility

Please notify Ali if you require a sterile cell sort. It is impossible to sterilize instrument 100% but we can take precautions to minimize any potential contamination of the sorted populations. We highly recommend the use of antibiotics if sorted cells are to be cultured following cell sorting.

Collection of Sorted Cells

The sorted cells will be diluted in sheath fluid. We routinely use a sterile sheath buffer, a high-quality PBS solution that is endotoxin-free and preservative-free from Dako. We can sort into polypropylene tubes, microscope slides, or multiwell tissue culture plates. In all cases, it is best to sort into vessels with FBS/medium/antibiotics to help cushion cell deposition.

Post-Sort Purity

The purity of each sorted population can be tested after each sort if desired.. The purity of each sort can be compromised by multiple factors including sample characteristics, other than instrument failure.

- The cells of interest are not completely resolved from the unwanted cells (overlapping populations)
- Unwanted cells (or platelets/debris) may be aggregating with cells of interest. These unwanted particles can fall off after the sort, resulting in the contamination by a number of unwanted particles
- Loss of viability after the sort may result in cells exhibiting different light scatter properties from those of the original cells.
- The capping of cell surface markers after the sort can result in sorted cells that are somewhat less fluorescent than the original selected population.

Cell Viability

We have found that post-sort handling can easily lead to low cell recovery. In order to maximize post-sort cell recovery and viability by some simple procedures, we recommend sorting into 1 ml of FBS and the use of centrifugal forces of 1500 x *g*.

Contact Information

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