

FLOW CYTOMETRY and CELL SORTING FACILITY

Department of Pathobiology

Room 250A Greene Hall

(334) 844-2711

DATE: \_\_\_\_\_

SUBMISSION FORM

\*Please fill out as completely as possible.

Submitted by: \_\_\_\_\_ Email: \_\_\_\_\_

Dept: \_\_\_\_\_ Phone#: \_\_\_\_\_

Charged to: \_\_\_\_\_

What exactly do you want to measure?

- Analysis
- Cell Sorting

What kind of information are you looking for? (i.e. Surface markers, DNA content, 1 or more colors, 4 way sorting, sorting to 96 well culture plates)

Type of sample(s) submitted: (i.e. Cell type/source/species)

Any special preparation (e.g. enrichment, deletion of certain populations, transfection, etc.)

Dye(s) used (i.e. PE, FITC):

Wavelength(s) you are interested in:

Emission Excitation: 488 (blue) 635 (red) 405 nm (violet)

Are your cells less than 70 microns in diameter?

How many samples are you submitting?

Is your sample fixed (rendered harmless)? Biohazard level: \_\_\_\_\_

No human cell lines without prior permission. You must be approved by the Auburn University's Biosafety Committee if you are from an outside organization.

Please do not submit samples that you do not have institutional approval for. No unfixed human blood samples.

- Is your sample TOXIC? (i.e. formaldehyde, propidium iodide)

Have you included all the necessary CONTROLS?

- Unlabelled cells
- Isotype controls for each antibody if appropriate
- Single label controls for multi-labeled specimens
- We recommend a viability marker ie. Zombie dyes, Ghost dyes or PI
- We recommend a post sort analysis of sorted populations

Please list your controls:

Approximate volume/cell concentration?

What are your cells suspended in? (i.e. Hanks with 2% FBS, PBS, etc.)

The cells were filtered to:

- 20 um
- 50 um
- 70 um
- other

Special Requirements? i.e. Sterile, Purity, Recovery

GUIDELINES:

1. Samples must be in 12x75 mm polypropylene test tubes (Falcon #2063) or 1.5 ml eppendorf or 0.5 ml eppendorf or 15 ml pointed centrifuge tubes or 50 ml centrifuge tubes (Please check to see if they fit the holder for the SmartSampler)
2. ALL samples must be filtered. See website for suggestions.
3. Minimum sample volumes are 0.5 ml. However if you have only 300 ul and a previous protocol set up it can be managed.
4. If you have samples stained with more than one fluorochrome per tube, please bring in a sample stained with each fluorochrome individually. This is for compensation purposes.
5. If you are sorting, cells should be concentrated – 5 to 10 million cells per ml and FILTERED. Lower concentrations can be sorted but will take additional time. Collection tubes for 4 way sorts should be 12x75 Falcon polypropylene tubes with interiors coated with 0.5 ml of 100% FBS. The FBS reduces cell adherence to the

walls of the tubes when sorted and keeps the cells HAPPY when they are diluted with sheath fluid. If you are having cells sorted, be prepared to collect them promptly. Sorting dilutes cells greatly so sufficient numbers of tubes must be prepared to collect the sorted cells.

6. For two way sorts either 15 or 50 ml tubes can be used. For plate sorts please provide an empty plate of the same type being used for the plate sort in order to set up an exact template.
7. Yields of sorted cells are proportional to the % of the population you are sorting for. The time for sorts is proportional to the purity/enrichment desired for that population. If you count your cells immediately prior to sorting the efficiency of yield can be estimated and may also help trace problems associated with low yield.
8. Tubes with visible cell clumping/debris will not be run.
9. The MoFlow is equipped with a temperature controlled sample chamber and collection holder to keep both the pre- and post-sorted cells at a constant temperature. If you would like to use this feature please let the operator know in advance of the sort to equilibrate the equipment to the desired temperature.
10. Tube changes during the sort (both sample and collection) provide opportunities for contamination. Please use antibiotics subsequent to the sort if you are culturing your cells
11. Please do not attempt to sort after 3 pm on Fridays. There is not enough time for involved sorting and cleaning of the machine after 3 pm on Fridays.

DATE: \_\_\_\_\_ Expt Name: \_\_\_\_\_

Person Submitting: \_\_\_\_\_ Phone: \_\_\_\_\_ email: \_\_\_\_\_

Tube #	Sample information/conditions	Fluorochrome/probe	Control
1	Unstained cells	NONE	AutoFlourescence control
2			
3			
4			
5			
6			
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