FLOW CYTOMETRY CORE FACILITY

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CELL SORTING GUIDELINES

The current setup for the cell sorter (FACSAria), which has no containment system for aerosols, prevents us from running samples that contain radioactivity or potentially infectious agents to humans.

- Cells for sorting must be brought in Falcon 12x75mm polypropylene test tubes (Falcon #2063, VWR 60819-728).
- Please filter your samples right before you bring them to be sorted. If the cytometer clogs you can lose all of your sort time not to mention cause expensive damage to the sorter. **Use Falcon 12 x 75 mm polystyrene test tubes to filter (Falcon #2235).**
- Minimum sample volumes are 0.5 ml.
- **Bring controls. To make conclusions about your experiment, the proper controls are critical!!!**
    - A negative control is an unstained sample or a sample stained only with the secondary.
    - Single color controls. If you have samples stained with more than one fluorochrome per test tube, bring in a control sample stained with each fluorochrome individually. This is for compensation purposes. DO NOT add PI to your single stained controls.
        - Ex. of controls for a simple GFP experiment:
            - negative control = cells not expressing GFP
        - Ex. of controls for a dual Fitc PE Experiment
            - unlabeled control
            - FITC only control
            - PE only control
- Cells should be concentrated to 50-100 million cells/ml for the high speed sorters. Note that these concentrations are optimal; lower concentrations can be run on the machines at slower rates although the additional time needed is billed normally.
    - Ex. Lymphocytes – concentrate 50 million cells/ml
    - Ex. Sticky/Adherent cells – concentrate 20 million cells/ml
- Use the appropriate media to sort the cells into. Pre-coat your collection tubes with BSA for better cell recovery. You should put about 1 ml of media or serum in the collection tubes and bring a couple of extra tubes in case there is a clog.
- Use appropriate size collection tubes. Cells will be given back to you at a concentration of about 500,000/ml. Any brand of collection tube is fine to use.
    - Ex. If you expect 3 million cells use 5 ml or 15 ml tubes
    - Ex. If you expect 100,000 cells use a 1.5 ml eppendorf tube

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 in the culture media if sorted cells are to be cultured following cell sorting.