CELL SORTING GUIDELINES

The cell sorting flow cytometers can not run samples that contain radioactivity or potentially infectious agents to humans. This is because these flow cytometers produce aerosols, and there is no containment system designed within them.

- Follow the New User Instructions on our web page for how to access our services. A fully completed new user form must be on file before we accept any sorting appointments.

- 24 hour advanced notice for a cancellation is required to avoid the full charge for sorting.

- Cells for sorting must be brought in Falcon 12x75mm polypropylene test tubes (Falcon #2063, VWR 60819-728) **. This tube is different then the analyzer tubes.** These tubes can be purchased in the on-campus VWR stockroom.

- 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).** A filter is built into blue cap. Gently put your pipet tip on the filter and push cells through strainer into tube. Make sure to transfer cells to the polypropylene tubes after filtering.

- Minimum sample volumes are 0.5 ml. Maximum sample volume is 3.5ml.

- **Bring controls. To make conclusions about your experiment, the proper controls are critical!!!**
  - A negative control is an unstained sample.
  - Secondary controls are useful to set the proper gating on negative and positive populations.
  - Single color controls. If you have samples stained with more than one fluorophore per test tube, bring in a control sample stained with each fluorophore 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. Note that these concentrations are optimal; lower concentrations can be run on the machines at slower rates.
  - Ex. Lymphocytes – concentrate 50 million cells/ml
  - Ex. Sticky/Adherent cells – concentrate 20 million cells/ml

- Use appropriate size collection tubes. Cells will be given back to you at a concentration of about 500,000/ml PBS. 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

- Use the appropriate media to sort the cells into. Precoat your collection tubes with 100% FBS or 5% BSA for better cell recovery. You should put about 1 ml of media + 20% serum or 1 ml of 100% serum in the collection tubes. Bring a couple of extra tubes in case there is a clog.

- **ALWAYS** put antibiotics in your culture media. Cells are sorted in an aseptic, not sterile environment. Our sorters are routinely cleaned. We would appreciate knowing which sorter caused the contamination if you know you got it from our instrument.

- We may have to cancel your sort if the sorter operator is sick or if they can not get into work because of inclement weather. Cancellations may also occur if the flow cytometer breaks down. We will do our best to get your sort in by working through lunch etc., but please be aware staff are not paid to work overtime. If MIT closes for the day, staff are not expect to come into work. If MIT closes early, staff are not expected to stay after closing. Fortunately these situations do not occur frequently but your understanding is appreciated if you become affected by circumstances that are beyond our control.