SWANSON BIOTECHNOLOGY CENTER AT THE KOCH INSTITUTE Flow Cytometry

Sorter Training Guidelines

Due to aerosolization, there is no sorting of radioactive or samples with known BL-2 pathogens that can be transmitted by mucous membrane.

You must bring samples for training.

- Cells must be in a **Falcon 12x75mm** polypropylene test tube.
- Samples must be in single cell suspension. Use a microscope during the dissociation step to ensure single cell suspension.
- Use 1 mM EDTA if sticky cells
- Use 10 units DNAse/ml if lots of dead cells
- Minimum cell volume is 0.5 ml.
- Maximum sample volume is 3.5 ml.
- Concentrate cells to optimal #'s

  Lymphocytes-concentrate 10-30 million cells/ml
  Cancer cell lines-concentrate 5-10 million cells/ml
  *For other cell lines reach out to the Flow Core staff

- Washing step/antibody titration. Resolves populations and leads to better data.
- Resuspend cells in PBS or HBSS. Bring extra buffer if you need to dilute your cells or if there is a clog.
- **ALWAYS** put antibiotics in your culture media. Cells are sorted in an aseptic not sterile environment.
- Bring collection tubes precoated w/100% FBS or 2% BSA. Add 1ml of 100% FBS or 2% BSA to sort cells into.
- Sorted cells come out at a concentration of 500K per ml of PBS. Use appropriate size collection tubes for the expected number of cells to be deposited into your collection tube. Example, if you're sorting 100K cells, you want to sort into a 1.5 ml Eppendorf tube. Up to 1.5 million cells sort into a 5 ml tube. Up to 5 million cells sort into a 15 ml collection tube.
- For plate sorting: 100ul media into each well. Ideally, 50% conditioned media and 50% fresh.

Always filter your cells! Clogs cost time and money.

- Use the blue cap from Falcon #352235. Add to a polypropylene test tube and filter to remove clumps. Gently put pipet tip on filter and push cells through strainer into tube.

- For cell anti-aggregation reagents, try Pluronic F-68 non-toxic, non-ionic surfactant. It is commonly used in bio-fermenters and I have found it to keep various concentrated samples of primary cells and cell lines from forming aggregates during very long sorts. Also works very well for hepatocytes, IEL's, CHO, fibroblasts, and many other cell types having a propensity to form aggregates. It also works well for analyzing and sorting mixed phytoplankton strains from sea water.
    - Pluronic is sold by Sigma, Cat# P556 as a 10% sterile, stock solution in a 100mL bottle. Add to your sample to make a final concentration of 1% vol/vol.

- Accutase and Accumax from Innovative Cell Technologies in San Diego, CA. A description of each product can be found at the links below:

  http://www.innovativecelltech.com/accutase.html
  http://www.innovativecelltech.com/accumax.html

Bring proper controls. Without the proper controls, we cannot setup your experiment template correctly.

- **Prepare the following controls for every experiment = Do not reuse compensation**.
    - **Unstained**-same cell type as samples without any fluorophores or viability dyes added. *Needed for autofluorescence.*
    - **Single color**-Bring one for each fluorophore and viability dye in your experiment. *Needed for spectral overlap (compensation).*
    - **FMO**-All fluorophores minus one. *Important for defining positivity of a population for the omitted color.*
- If you use secondary antibodies, you must bring a sample labeled with secondary only.

Your FACS DIVA login account and template are instrument specific.

- If you need an account on another instrument, submit the Request for DIVA Account

- If you need a template on another instrument, follow the “How to DIVA” document found in the training materials. You can also reserve time with a Staff member for guidance on creating your template.

- Transfer your data at the time of your reservation. If you do not you will be billed a minimum of 30 minutes once you log into DIVA.