
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 polystyrene** test tube. No substitutions. No polypropylene tubes. No VWR or other manufacturer tubes.
- 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 of 50-100 million cells/ml (Lower concentrations can be run at slower rates)
 - Lymphocytes-concentrate 50 million cells/ml
 - Sticky/Adherent cells-concentrate 20 million cells/ml
- Washing step/antibody titration. Resolves populations and leads to better data.
- Resuspend cells in media or PBS with protein such as BSA. 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 5% BSA. Use 1ml of media + 20% serum or 1ml of 100% serum.

Always filter your cells! Clogs cost time and money.

- Use Falcon 12 x 75 mm polystyrene test tubes to filter (Falcon #352235, VWR # 21008-948) cells to remove clumps. Filter is built into blue cap. Gently put pipet tip on filter and push cells through strainer into tube.
- Filter plate
 - Millipore 40UM Nylon Mesh
Clear Baseplate 2 PK
Cat: MANMN4010
Lot:ROHA36763
- 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

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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 dim populations and gating*
- If you use secondary antibodies, you must bring a sample labeled with secondary only.

Your 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 Analyzer Training video linked below. You can also reserve time with a Staff member for guidance on creating your template.
http://web.mit.edu/ist-train/Koch_Analyzer/#/
- Transfer your data at the time of your reservation. If you do not you **MUST** make a reservation on the instrument, and you will be billed a minimum of 30 minutes.

