Preparation for Aseptic Sorting in the Flow Cytometry Lab

1. Principle

1.1. The FACSArias in the Stanford Stem Cell Institute are used for sorting of cells for downstream applications. These applications may include injection into animals, culturing, and RNA analysis of single cells, all of which are negatively impacted if cells or contaminants from previous sorts are not cleaned adequately from the sample tubing and sort areas. This protocol is designed to describe how users will clean the FACSArias before and after use to prevent contamination between sorts. The flow cytometry manager cleans the sheath path with 70% ethanol on a weekly basis or as necessary. Sheath tanks are replaced with freshly autoclaved tanks as necessary.

2. Materials and Equipment

2.1. Laboratory coat or gown.
2.2. Gloves.
2.3. Eye protection (Safety glasses or Safety goggles)
2.4. Appropriate disinfectant (10% bleach or Cavicide.)
2.5. FACSClean (preserved 10% bleach)
2.6. FACSRinse (rinsing solution)
2.7. Sterile water and/or sterile staining buffer.
2.8. 70% ethanol
2.9. Kimwipes and paper towels.
2.10. Sterile (fresh) CST and Accudrop beads.

3. Procedure

3.1. Wear necessary PPE (refer to Table 1 in SOP.002, Biohazard Containment in the Flow Cytometry Laboratory, for required PPE.)
3.2. Prior to turning on stream, wipe down all areas inside the sort block with Cavicide. Remove plates for better access and clean them with Cavicide also. Dry surfaces and plates before replacing.
Wipe down the collection device to be used with Cavicide. Open the drawer and wipe that area with Cavicide. Wipe down the sort chamber with Cavicide. Wipe down the sample loading area with Cavicide.

3.3. Clean your selected nozzle by sonication in DI water if necessary. Check the cleaning has removed any salt deposits, then rinse the nozzle with 70% ethanol and dry. Wipe the nozzle insertion area with a q-tip soaked in 70% ethanol. Insert the nozzle.

3.4. Set up the instrument as usual for your nozzle and start the stream. Check the instrument performance with CST beads and do the Drop delay setup with Accudrop beads.

3.5. Prior to placing your cells on the sorter, clean the sample path by running 10% bleach (FACSClean) as a sample at a flow rate of 11 for 10 minutes.

3.6. Follow this with 1 minute of FACSRinse.

3.7. Follow this with 5 minutes of Sterile Distilled water. Note: This water should be sterile, aliquoted in the hood. You can also use your sterile staining buffer for this rinse step, and it may be helpful to rinse for longer if your cells are particularly sensitive to any residual cleaning fluids.

3.8. Back flush the sample line for 1 minute.

3.9. The system is now clean and your cells may be run. Note that if you are using Comp beads, or any other reagents, these should be prepared sterile and not used for multiple experiments. If there is a problem with the stream that necessitates accudrop beads to be rerun, those should be prepared sterile, or the sample line must be cleaned again after their use as in steps 3.5-3.8 above.

3.10. When you finish an experiment, the instrument must be cleaned thoroughly of your cells and any contaminants introduced with them. Clean the sample path by running 10% bleach (FACSClean) as a sample at a flow rate of 11 for 10 minutes.

3.11. Follow this with 1 minute of FACSRinse.

3.12. Follow this with 1 minute of distilled water.

3.13. Turn off the stream and remove the nozzle.


3.15. Clean the flow cell with distilled water.
3.16. Wipe down the surfaces of the instrument as described in step 3.2 above.
3.17. Wipe down all table surfaces with 10% bleach.