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Using a Sonicator Bath to Clean the Nozzle on a BD FACS Aria II/III/Fusion, SONY SY3200, or Other Instrumentation with Similarly Sized Nozzles

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Flow Cytometry

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ABSTRACT

Sonicating the nozzle is an important step when dealing with clogs on a cell sorter. In sorters where the nozzle positioning is not fixed, such as a Sony SY3200, sonicating and removing the nozzle may not be as frequent of an occurrence. However, a safe and effective protocol to clean the nozzle remains very important. On instrumentation like the Aria II/III/Fusion, sonicating the nozzle can be a daily occurrence as it needs to be done before the stream is started, and it may need to be done in the middle of a sort if the sample clogs during the run.

Certain precautions need to be taken during this process if there is a need to remove a clog caused by a BSL2 or higher sample. The safety of the instrument operator is of the utmost importance during this process. Additionally, most institutions require SOPs for this process which take biosafety into account. This protocol for sonicating takes operator safety, as well as institutional requirements into account, while providing an effective method of cleaning the nozzle or removing a sample clog without causing damage to the nozzle.

GUIDELINES

Do not use tap water for any of the steps that require water. Always use DI water from a MilliQ or similar water dispenser.

Keywords: flow cytometry, cell sorting, biosafety, BSL2, BSL1, BSL2+, cleaning, best practices

MATERIALS

12x75 polypropylene or polystyrene FACS Tubes. No cap: BD 352008 or With cap: BD 352054
Parafilm
70% Ethanol
CaviWipes or 25% Bleach
DI Water
Hemostats or Floating Foam Tube Rack
Sonicator Bath
KimWipes
Paper Towels
Gloves

SAFETY WARNINGS

Make sure to wear gloves and appropriate PPE throughout the process. Sorters generate aerosols, and clearing a clog can be one of the most potentially hazardous points in a sort. Make sure to clean the sort block (if applicable on your sorter), deflection plates, and biosafety cabinet well with 70% ethanol, CaviWipes, or 25% bleach if you experience a clog.

Do not use 2.5mL of neat or diluted bleach to place in the FACS tube where the nozzle will be submerged if you are sonicating a BD FACS Aria II/III/Fusion nozzle, as it will degrade the material. BD recommends DI water to sonicate the nozzle when possible (i.e. when it is not clogged with a BSL2 or higher sample). Instead, use 70% ethanol as the recommended solution when the sample is BSL2 or higher.

BEFORE START INSTRUCTIONS

Prepare at least 100mL of 70% ethanol before the sort.

1 Wipe down the interior of the sonicator bath with a CaviWipe or 70% ethanol

2 Check that the sonicator is connected and the power is on.

3 Very carefully fill the sonicator bath to operating line with DI water from MilliQ or similar dispenser.
Do not use regular tap water.
Do not fill a small receptacle in lieu of the sonicator bath as the sonicator operates optimally and safely when filled to the operating level and can be damaged if it is not filled properly.

4 Perform this task inside the biosafety cabinet.
When working with a BSL1 sample, place the nozzle in a 5mL FACS tube along with at least 2.5 mL of DI water to ensure that the nozzle is fully submerged. Cover the tube with a cap or with parafilm.

4.1 If using a BSL2 or higher sample, use 2.5mL of 70% Ethanol instead of DI water in the tube. Cover the tube with a cap or with parafilm. Again, be sure to work with any BSL2 or higher sample within the biosafety cabinet before proceeding.

5 Take the tube to the sonicator bath. Depending on the location of your sonicator bath, you may need to remove the tube from the biosafety cabinet, which is why covering the tube is important.

6 Using a pair of hemostats, lower the FACS tube with the nozzle into the sonicator bath.
Alternatively, a floating foam tube rack can be used to hold the tube safely in place in the bath.
Do not allow the tube to touch any part of the metal surface of the bath.
Lowering the tube with a gloved hand is not recommended but can be done because sonication produces heat which will cause discomfort.

7 Sonicate for 30 seconds.

8 After 30 seconds, remove the tube from the sonicator bath.

9 In the biosafety cabinet, prepare a paper towel with a lint-free KimWipe on top. Move the tube into the biosafety cabinet and remove the cap or parafilm.

10 Spill the contents of the FACS tube, including the nozzle onto it. Fold it over and gently pat the nozzle dry.

11 Insert the nozzle back into the instrument.

12 Discard the paper towel and KimWipe into a biohazard step can or bag. Discard the empty tube in the appropriate sharps container. Wipe down the area with a CaviWipe or 70% Ethanol.

13 Drain the sonicator bath at the end of the day: disconnect the power cable from the bath and pour the DI water into the sink. Dry the interior and exterior of the bath with a paper towel to prevent rust formation, biofilm formation, or other damage to the device.