Bauer Core Standard Protocol		
Title: Becton Dickinson FACs Aria III Protocol		
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1. Purpose

This protocol provides instructions for running samples on the BD FACs Aria III.

2. Materials

Filtered samples
Collection tubes or plate
IsoFlow Sheath Fluid (Beckman Coulter #8546859)
Bleach
Distilled Water

PREPARING THE INSTRUMENT

1. Log on to computer with research computing account (account used to sign up for time on Spinal) if not currently logged in
2. Turn on the FACS Aria by pressing green power button on the side.
3. Fill sheath tank with IsoFlow Sheath Fluid and empty waste in the sink, pouring 500mL of bleach in the waste container after emptying.
4. On the desktop find BD FACs Diva, right click the icon and click “run as administrator”. When the software starts set the user to “Administrator” and hit ok. The software will take a moment to connect to instrument. When it is completed the software will display a message reading “CST Mismatch” click the middle button “Use CST settings”.
5. Open the Coherent Connection and turn off the appropriate laser based on the lasers required (405nm is the default laser on Aria #1)
    a. To use the 355nm laser on Aria #2 press “Stop” and then “Start”
    b. All lasers are on by default
6. On the FACS Aria wet cart:
    a. Reconnect the fluid line to the filter (only sheath fluid should run through the filter) (fluid line connects to the blue line).
    b. Reconnect the pressure line (pressure line connects directly to the sheath tank).
        i. You can check the pressure gauge on the sheath tank or on the side of the sorter. (It 80psi for startups, but it will decrease if you are using a different nozzle. 70 nozzle = 70psi, 85 nozzle = 45psi, 100 nozzle = 24psi, 130 nozzle = 12psi. You can also unscrew sheath tank and if the lid doesn’t fall in you know it is pressurized).
    c. Make sure the yellow line (air supply) on the side of the fluidics cart has the blue valve open by pointing it towards the instrument.

7. Make new bleach tubes each day (label and date)
8. Verify what nozzle is being currently used, change if necessary.
    a. Change the nozzle by going to Cytometer in toolbar and choosing View Configuration, a new window will pop up with the different nozzle and laser selections. Highlight the nozzle and laser set up required and click "Set Configuration".
        i. This will ask you to verify the mirror and filters hit okay once satisfied.
    b. Select ok at the bottom right and then exit out of the current page.
    c. Upon returning to the DIVA software a message will pop up saying CST Mismatch. Select the middle option "Use CST settings" this will ensure the correct nozzle settings.

PERFORMING FLUIDICS START UP

1. The Start is required if the fluidic shutdown has been done. To verify the bottom right of the software says, "Fluidic Startup/Shutdown Done". If Startup has already been completed feel free to move to "Starting the Stream". Proceeding with an additional startup will not hurt the instrument.
2. From the BD FACSDiva Cytometer menu, select "Fluidics Startup". This sets the pressure and fills the fluidic lines. A window appears that will walk you through the fluidics start up procedure below.
    a. Verify that the air and fluid lines are disconnected from the ethanol tank and connected to the sheath tank and out of the way (if they are disturbed while stream is on it can affect the stream), then click Done.
    b. Verify that the closed-loop nozzle is installed in the flow cell, then click Done.
    c. Remove the closed-loop nozzle from the flow cell, then click Done.
    d. Insert the correct nozzle size in the flow cell. Nozzle size is on end.
        i. Make sure the top side of the nozzle is facing up as you insert the nozzle.
        ii. Push the nozzle all the way back into the flow cell.
        iii. Turn the nozzle-locking lever clockwise to the 12:00 position.
        iv. If changing to a nozzle that needs less pressure bleed the sheath tank of pressure.
    e. Click OK to complete the process.

STARTING AND SETTING UP THE STREAM

1. Start the stream
    a. Click the sorting button (bubbling test tubes) on the workspace toolbar to display the breakoff and side stream windows. (This should already be open).
    b. Click the stream button (the red x) in the break off window to turn on the stream.
2. Open the sort block door and check the stream.
    a. Stream should flow smoothly from the nozzle into the center of the waste aspirator.
    b. If stream is flowing but is unsteady check for bubbles in the flow cell. If you see bubbles, turn of the stream, wait for 10 seconds, and turn on the stream again.
    c. If you see dripping or spraying, or the stream image appears abnormal, turn off

the stream and troubleshoot.

i. If steam not directly into middle of the waste drawer than unscrew the two screws to the right and left of the flow cell, gently move the whole apparatus until the stream is in the middle of the waste drawer and tighten the screws to hold it in place. DO NOT OVERTIGHTEN THE SCREWS. This can damage the flow cell.
ii. Can remove pin and find any liquid out of place. Use KIM wipe or Q tip to dry all areas. Any liquid on any of the surfaces will affect the stream.

3. Close the sort block door.
4. Setting Up the Breakoff
    a. Once you see the black breakoff bar in the stream window go ahead and hit "Sweet Spot"
        i. Verify that the small satellite droplets are merging with the large droplets
            1. Satellite drops should merge into the drops in 6 satellites or fewer, if it isn't you may need to remove and reinstall the nozzle or sonicate it.
        ii. Ensure that the Drop 1 value given and last set are similar.
            1. Once a valid target had been established, you do not need to reset it unless you change the nozzle or you sort setup option
            2. If the target Drop value causes the amplitude to exceed 70 volts you need to restart the stream and try to find the Drop 1 value by adjusting the amplitude.
        iii. Do not want the gap to be too low but it also should not be more than 15. A good rule of thumb here is ~10.
            1. If the gap is more than 3 away from the set point the sort will pause.
        iv. Cannot have the sweet spot on for CST. If you try to go into the CST software with the sweet spot on it will give you an error. (It will just tell you to shut it off).

CLEANING THE SYSTEM

**** Note ** Take the lid off ALL tubes before loading onto the sorter!!!**

1. Start new experiment.
2. Open new specimen (Syringe). This will automatically give you a tube.
3. Click on the syringe + to open and click the green pointer arrow next to the tube. This will allow you to load the tube.
4. Place the new bleach tube into the sorter.
5. Press load.
6. Let the bleach run through the sorter for 10 minutes at a speed of 3 (minimum).
7. Press the unload button.
8. Remove the bleach from the sorter.
9. Add new water tube to the sorter.
10. Press load.
11. Let the water run through the sorter for 10 minutes (minimum).

CHECKING CYTOMETER PERFORMANCE

1. Turn off the Sweet Spot.
2. Select Cytometer → CST.
3. Verify the bead lot information under Setup Beads matches the CST bead lot.
4. Verify the cytometer configuration is correct for your experiment.
5. Retrieve the beads from the fridge.
6. If no beads prepare the beads by adding 1 drop of beads to 5-6 drops of dH2O.
7. Install the bead tube onto the cytometer loading port.
8. In the set up control window select Check performance from the characterize menu
9. Click Run
10. Verify that the performance check has passed
11. Once the performance check is complete click view report. Make sure you compare the reports from the same nozzle size. Should open that way in the report window in CST.
12. Close the CST window to connect back to DIVA.
    a. Click use CST settings in dialog box that appears.
13. Delete the experiment.

ACCUDROP

1. Turn on the Sweet Spot.
2. Make sure the stream is steady and not jumping around. (If it is jumping around make sure the Drop 1 value is correct. If that is not the issue restart the stream and attempt again)
3. Bring the slider bars for the two inner sort streams to 35, tune the micrometer to make the waste (center) drop large and bright
4. Double click to open the Accudrop experiment and open the tube
5. Click the plus on the global worksheet and double click to open the sort layout
6. Load Accudrop beads onto the sorter and acquire data
7. Once the beads appear in the plots click sort and then cancel
8. On the sort stream viewer press voltage and then optical filter
9. The left box should greater than 95%
    a. If the value is less than 95% manually toggle the drop delay values up or down depending on the corresponding changes.
    b. You can also click auto delay and the instrument toggle through drop delays until an ideal one is found
    c. Make sure the beads are running greater than 1000 events/s, increase pressure or concentration if this is not the case
    d. Make sure the sort streams are nice and bright by adjusting the micrometer
10. When greater than 95% unload the tube and proceed to start your experiment

SHUTDOWN PROCEDURE

1. After finishing samples, run Bleach as a sample on a speed of 3.0 for 5 minutes
2. Run dH2O for 1 minute at a speed of 3.0
3. Press unload but keep the dH2O tube in sample holder

4. Shut off stream by pressing “Red X”
5. Remove nozzle and insert closed loop nozzle
6. Ensure there is at least 2mLs of dH2O in tube
7. From the top menu select “Cytometer” -> “Clean Flow Cell”
    a. Click OK on popup to commence cleaning, when completed hit OK
8. Quit Diva and coherent connection
9. Spray down workstation if dealing with BL2 materials
10. Power off Aria by pressing green power button on the side
    a. Turn Chiller off if it was in use
11. Close pressure gage by turning Blue Plastic away from the machine

BIOSAFETY

1. Closed toed shoes and clothing that goes below the knee is required at all times in the lab
2. BL2 samples can only be ran on Aria #2
3. Lab coat and gloves are required for BL2 samples and chemicals
4. Goggles must be worn in addition to a lab coat when pouring chemicals greater than 500mL
    a. Chemicals cannot be disposed of down the sink
5. Immediate action following a clog when running BL2 samples is turn on the whisper HEPA filtration system which is located under the bench
    a. Power switch is located on the bottom right of the whisper unit
    b. Do not open the sort door down until all aerosols have been evacuated (1 minute)
6. Instrument cleanup is required after running BL2 samples which consists of 10 minutes of bleach followed by 10 minutes of water
7. Spray down the workstation with 70% ethanol when finished with the machine