

# AMREP FLOW - LSR II users SOP

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## Important Points

- **Please do not download any program or applications from the internet (eg iTunes, Facebook Apps etc) on to LSR II computer. This is important as these programs can begin automatic updates which can potentially disable the DIVA software**
- Any questions please ask a member of the AMREPFlow Staff – if problems arise which you are unsure of please do not try and fix yourself.
- All users must be licensed to use the instrument and be familiar with the AMREP Flow Department policies. Disregard may result in suspended use of instrumentation. To become licensed please contact a member of the AMREPFlow Staff.
- Correct shutdown procedure must be followed. Failure to follow thoroughly can result in instrument malfunction thus the next user being affected.

## Sample Preparation

- ALL samples MUST be filtered through a 70um mesh filter before use – NO EXCEPTIONS.
- All samples must be at an appropriate cell concentration in order not to block the Sample Injection Probe (SIP).
  - Recommended cell concentration is  $1 \times 10^6$  cells / ml ( $2 \times 10^5$  cells / 200 $\mu$ l)
- Users who continue to block the SIP through neglectful sample preparation may have their usage privileges withdrawn.
- The LSRII contains a 405nm, 488nm and 633nm laser setup with the ability to detect signals in 15 channels. Before designing an experiment or purchasing an antibody, please ensure it is compatible with this instrument. Instrument capabilities are available at [www.amrepflow.org.au](http://www.amrepflow.org.au)

## Instrument Start-up

1. Switch on the LSRII at the front of the instrument. This will warm up the lasers and pressurise the fluidics.
2. Switch on the Computer.
3. Check the fluidics.

## Fluidics

The LSRII strictly uses MilliQ water as its sheath fluid. There are two 20L containers available in the lab, located next to the sink.

The Sheath Tank is the stainless steel container. It must only be filled with MilliQ water to  $\frac{3}{4}$  of the tanks total volume (to the seam line). This should be enough fluid to run the instrument for at least 6 hours. However, as there is no sensor to indicate the volume remaining, users must check the level before each session by opening it and looking.

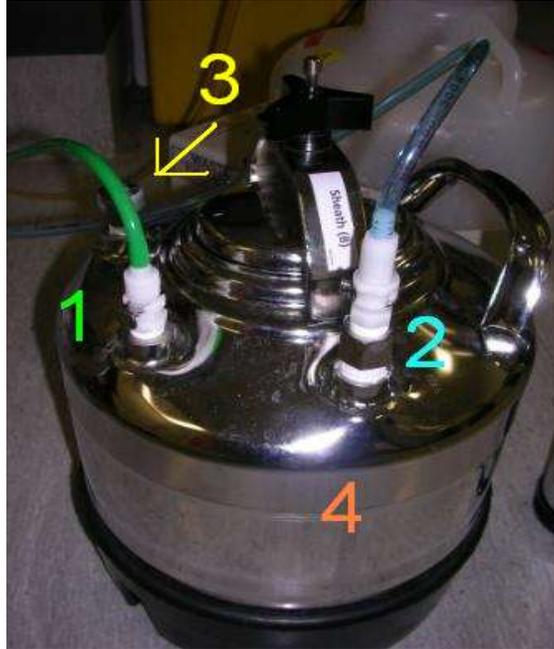


Diagram 1 – Sheath Tank

To fill the sheath tank:

- a) Remove the pressure tube from the tank. (1)
- b) Remove the fluid tube from the sheath tank. (2)
- c) Pull up the pressure valve for a few seconds until all pressure has been relieved. (3)
- d) Unscrew the top of the tank and removed the lid. Fill with MilliQ water to the metal seam in the tank. (4) This will ensure the tank is roughly  $\frac{3}{4}$  full.
- e) Replace the lid - it will only fit in one way – and tighten screw ensuring that the metal handle is in line with the sheath port (2) as shown in Diagram 1. Do not force the lid or handle in any other way.
- f) Reconnect the sheath tube (2) and the pressure tube (1). Do not force them as they are designed to only connect to the correct valve. Wait 30 seconds until the sheath tank is pressurised, then bleed any air bubbles by removing the knob on the bubble filter and draining water into the bucket provided (5). This will expel sheath fluid, so ensure that any spills are wiped up.



Diagram 2 – Bubble Filter

**PLEASE NOTE:** The waste tank holds a much higher volume of fluid than the sheath tank. Do not use the waste tank volume as an indicator of sheath remaining. Periodically check the sheath tank volume to ensure the sheath does not run dry.

## **Waste Tank**

The waste tank will hold much more volume than the sheath tank. When the waste tank is full an alarm will sound and will not cease until the tank is empty, however this should not occur as all users should note that the sheath tanks holds a much smaller volume of fluid than the waste tank. At the end of your session please empty if the tank is more than 1/3 full of waste solution by following the steps below.

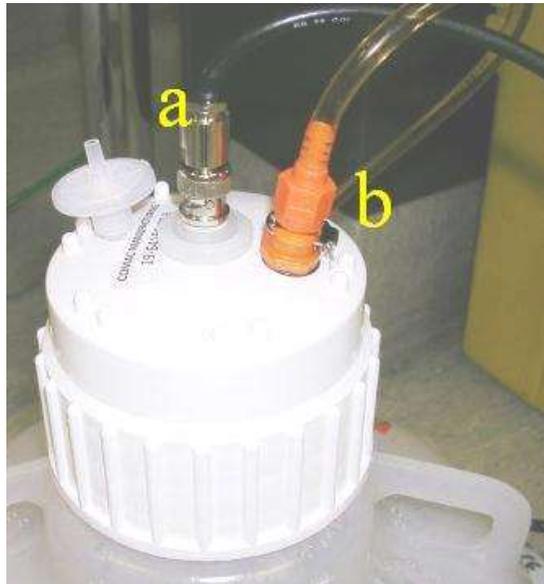


Diagram 3 – Waste Tank

To empty the waste tank:

1. Remove the black coaxial cable (a) by turning it anti-clockwise.
2. Remove the orange waste tube (b)
3. Pour the contents of the waste tank down the sink whilst running tap water at the same time to flush and dilute any excess bleach down the sink.
4. Once the tank is empty, add 10% Sodium Hypochlorite to the tank. Pour in between 100ml and 200ml. Please ensure you are wearing gloves and a lab coat for this step – Sodium Hypochlorite is hazardous and will irreversibly damage your clothing should you spill it on yourself.
5. Reconnect the tubing and (b) coaxial cable (c).

## **Software and sample setup**

1. Turn on the PC and both monitors. You will be prompted for a password which can be obtained from Geza or Michael
2. When logged in, click the BD FACS DIVA software icon.
3. You will be prompted for a username and password. When licensed, you will be given an individual login and password to use DIVA.

**\*\*UNDER NO CIRCUMSTANCES ARE LOGIN DETAILS TO BE SHARED. SHARING OF THESE DETAILS OR LOGGING IN FOR ANOTHER USER WILL NOT BE TOLERATED AND WILL RESULT IN YOUR ACCESS BEING LIMITED. STRICTLY LISCENSED USERS ONLY\*\***

4. Should you be prompted to use CST settings, select YES as these settings will use the most up to date time delay calibrations for the instrument. Should you not be asked to use CST settings, the instrument will already contain these settings.
5. From the SIP, remove the tube and prime the instrument twice using the button on the LSRII control panel. Place a tube of water on the SIP and press RUN from the panel with the speed setting on LOW
6. Adjust the SAMPLE FINE ADJUSTMENT knob to the middle setting. The knob is designed to make 10 complete rotations, thus in order to have it in the middle turn it all the way anticlockwise until it meets resistance, then turn it clockwise 5 complete rotations.

**FACS DIVA Software guide is available on LSRII computer desktop. Please refer to this document for comprehensive guidelines on how to use the software. If further assistance is required please see Geza or Michael.**

## **Instrument shutdown**

1. Post running of your final sample, move the support arm to the side, press RUN and allow at least 10 drops of fluid to fall from the SIP.
2. Place a tube of MilliQ water on the SIP and let it RUN at HI flow rate for 3 minutes, or 5 minutes if you are the last user of the day.
3. Repeat step 2 using 10% hypochlorite solution.
4. Repeat step 2 using MilliQ water.
5. Leave a tube containing 1 ml of MilliQ water on the SIP and set to STANDBY
6. Turn off cytometer and shut down the computer – the only time you should leave them on is if you have contacted the next user and you know they will be using the LSRII within an hour.

## **Troubleshooting**

The following is a list of the most frequently encountered problems. Please be familiar with these and attempt to recertify them yourself before contacting Geza or Michael.

### **“The Cytometer will not connect”**

This will be evident by everything being switched “on”, but DiVa software stating the cytometer is disconnected.

1. Switch the LSR II off. Wait 10 seconds. Switch the LSR II back on. If the LSR II has not connected after 2 minutes (be patient, connection can take this long!), try:
2. Switch the LSR II off and SHUTDOWN (not restart!) the computer. Wait a few minutes. Switch the LSR II and the computer back on. Start DiVa as normal. If the cytometer will not connect after a few minutes, see Geza or Michael.

### **“I’m not seeing events when I load my tube” / “There are no drops coming from the SIP when there is no tube on” / “My APC signal looks funny”**

This can be due to a number of factors. In this order try:

1. Adjust the O-ring at the top of the SIP so that it is tight enough to hold you tube independently of the supporting arm.
  2. Ensure your tube has no cracks – any breach in the pressure will result in your sample not being taken up by the SIP
  3. Ensure that the sheath tank is properly sealed; the tubing is correctly connected and has no kinks. Bleed all air out of the bubble filter.
  4. Ensure the “Fine Tune Adjustment” knob is not turned all the way down. Turn it clockwise several times if you are able to.
  5. With no tube on the SIP, move the supporting arm to the side and PRIME the instrument 3 times.
  6. Run bleach (available from the squeeze bottle next to the ARIA) for a few minutes on HI followed by water on HI.
- Still having issues? Please see Geza or Michael