Imune Defense Core (IDC) – Cell Analysis
Standard Operating Procedures
Location: BSE 3.108-1 on UTSA main campus

LSR-II cytometer

Notes for all users:

1. Only IDC experienced, trained and approved new users can operate the instrument independently. New users can arrange training with the Core Manager, Dr. Sandra Cardona and establish a LSRII user account.
2. All users need to sign an agreement form before the first time using any instrument in the core.
3. Be aware that IDC is a biosafety level 2 contaminant (BSL2) laboratory. All users require to follow University BSL2 laboratory policy.
4. Be on time and follow the cancellation policy.

Scheduling:

Schedule can be made via e-mail request to IDC.FLOW@UTSA.edu or trained users can request access to LSR-II google calendar. Schedule request is processed on first-come first-served basis. Please see after hour policy.

Cancellation policy:

Cancellations are allowed up to 2 hours prior to scheduled time.
A no show fee will be charged equivalent to one hour of usage for the reserved instrument.

LSRII start-up protocol (run by the core personnel):

1. Turn on workstation
2. Turn on the LSRII and wait for 30 minutes before logging in on the workstation and FACSDiva software. This procedure is to allow for proper boot-up of the instrument and stabilize the lasers. Please note that the users charge is based on the duration of running the FACSDiva program.
3. Perform daily startup procedure. Install a 5 ml polystyrene tube containing 3 ml of freshly made 10% bleach and run at high speed for 1 min with the sample arm on the side. Place the sample arm under the tube and continue to run for 5 min. Replace with a tube of MilliQ water and repeat the procedure.
4. Log on the workstation and login into the FACSDiva program. This requires an established account with IDC and a user name and matched password.
5. Check cytometer performance using the CS&T beads (BD Cat# 70106). Confirm the log number of the beads match with the information stored in FACSDiva program. If new log of beads is used, it requires to update the log information in the program.
6. If the instrument is passed CS&T test, turn it to standby. Please notify the core manager of any abnormality.
7. Exit the FACSDiva program.
Data Acquisition (run by approved researchers):

1. Please bring a sample submission form with your sample to the IDC laboratory. The form includes billing information that must be signed and approved by the responsible Principle Investigator.
2. Login to FACSDiva using your account name and password.
3. Check the CS&T status.
4. Run your samples
5. Export your experimental data and proceed to data storage procedure.
6. Logoff from the FACSDiva program first.

Data storage:

1. No USB drive or portal memory devices are allowed to connect to the workstation that operates the LSR-II.
2. Please save your acquisition templates and export your data to your PI’s folder in Z: drive. Create a subfolder inside your PI’s folder. The subfolder is named using the following system: Researcher initials followed by the date of the experiment with the following format YYMMDD (e.g. SMC180108). Please do not add spaces in the initials, but you can add letters at the end to specify your experiment if you prefer. The IDC server can be accessed from any computer on campus as follows:

   Open my computer
   Click on map network drive
   On folder type \cajal.utsa.edu\idcserver
   Click on connect using different credentials
   Click on finish
   Enter your CBI username and password (consult with your Principal Investigator) as follows: cbi.utsa.edu\username

   After you have saved your data right click on the Z drive and disconnect

3. If you don’t have an account to access the IDC server, please use an authorized computer in the core to transfer your data to USB drive, google drive, etc…
4. Data older than 3 months old will be deleted from the Z drive. IDC is not responsible for your data storage. Please backup your data after your experiment.

Cleaning/Shutdown procedure (run by approved researchers):

After finishing data acquisition, run a routine cleaning procedure to disinfect the instrument. Install a 5-ml polystyrene tube containing 3 ml of 10% bleach, run 1 min with the arm on the side and 5 min, the arm under the tube. Repeat the procedure using a tube of water. Place a clean tube with 1 ml of MilliQ water. Turn the cytometer to standby mode or turn off the power at the end of day.

Monthly maintenance (run by the core personnel):

1. Empty sheath fluid container and fill with 1 liter of 10% bleach
2. Install a tube with 3 ml of 10 bleach under the SIP.
3. Run at high speed for 1 min with the SIP arm on the side and continue for 30 min with the arm under the tube.
4. Empty sheath container. Examine the interior of the container for signs of erosion and clean.
5. Fill the sheath container with MilliQ water and place a tube of water under the SIP. Repeat step 3.
6. Empty the sheath container and clean. Fill the container with 6 litters of sheath solution.
7. Login to the LSRII maintenance form located next to the instrument.

Periodically maintenance and troubleshooting (run by core personnel):

1. This procedure is performed if the instrument does not pass CS&T test or quarterly.
2. Empty the sheath tank and fill with 2 litters of water.
3. Turn the instrument in standby mode. Install a tube of 10% bleach and run for 5 min with the SIP arm on the side. Replace with a water tube.
4. Place the arm back and press “Prime” twice.
5. Replace with 10 bleach tube, turn the instrument to run mode and run at high speed for 30 min with the arm under the tube.
6. Install a tube of water and repeat step 3-5.
7. Empty the sheath container and fill with sheath solution.
8. Install a tube of water and repeat step 3-5.
9. Run BD CS&T test and login to the maintenance book.

After hour policy:

Only approved personnel can operate independently after hour. All procedures must be followed and the researcher is also responsible for proper shutdown procedure as described above. Request access card to Core Manager in advance if necessary.

Report abnormality:

Please send the report to the core manager.

Contact information: Sandra M. Cardona, Ph.D.
BSE 3.108-1
Tel: 210-458-4089
IDC.FLOW@UTSA.edu