

# Apogee

Please use the more detailed manufacturer protocol until you are not familiar with the machine. This is only a short checklist to help you to remember the most important steps. Never use the machine without appropriate training.

## Before switching on the machine

Please do not use the machine if the temperature is below 15°C in the room.

Check fluid levels and prepare solutions if required

**Waste tank** must be empty. If not: Please ask the previous user to empty it as it may contain hazardous stuff. If previous user is not available handle waste as dangerous material. Check the connection on the top of the tank. *If it is not connected properly, the waste can flow back to the sample system and can damage the machine! There is no sensor in the tank, it can overflow as well.*

**Sheath fluid:** at least 2.5 l of sheath fluid must be in the machine before switching on. Extra tank can be connected if planning long measurement. Prepare an extra bottle of sheath fluid (2.5L MilliQ water +200uL ProClin 300), as it can run out easily during measurement. (It consumes about 1L per hour.)

*If run out, try "New sheath fluid". Sometimes it requires restarting of the machine. If still doesn't work, administrator log in would be required. After: "Remove air in syringe".*

**Cleaning solution tank:** Minimum 50 ml cleaning solution must be in it. (Cleaning fluid: 10 mL cleaning fluid (in the fridge) + 40 mL MQ.

## **Switch on machine and the computer**

Do not login until machine does not autocalibration (moving and blue flashing). After use Resercher login.

Start the Hitogram application. Be sure that you open it only once!!! If opened parallel it won't work.

**Please do not restart the machine without "Shutdown"!!!** Close histogram in task manager. If still doesn't work, administrator login may be needed.

Wait till the machine is ready to work.

## Calibration

If no calibration was done in the last month, make a bead measurement. Dilute T1527 beads 1:5 in ultrapure water and measure with the appropriate settings.

## Measurement

Settings depends on your experimental design. Set everything in control panel and autocycle for yourself or load a previously saved protocol. (**TRAINING!!!**)

### **Here are some reminders:**

- Data is saved automatically in a folder with the today's date.
- You can open saved setting for your measurement (File > Load settings). If you want to save protocols, save experiment to protocol folder, and you can open it later.

- If needs, switch on laser 638.
- Check if auto shutdown is on in Settings.
- Have at least 250 ul of samples in your Eppendorf, as the machine soak up at least 150ul and bubbles will interfere with measurement.
- If you think you need cleaning during your measurement use service tab in control panel.

### Shutdown procedure

Do not forget to save your results for yourself before shutting down the machine.

Cleaning: Run 10% bleach or Facs cleaning fluid (Cytotflex, blue solution) for 5 minutes. Run ultrapure water for at least 5 minutes. If event number is above 200 events/sec run a cleaning protocol (service tab), and maybe try new water, as solutions kept in eppendorfs can contain microplastic traces that can be detected by Apogee.

Close every running program on the windows except the histogram. Also remomve any of the extended hardver like pendrive before press shutdown button. It may couse software issues can be solved only in administrator mode and it takes days.

Press shutdown. It may last for 20 minutes for the machine to switch itself off.

Don't forget to empty the waste tank after yourself.

Further details in user manual and training!

Channel Parameters			
	Laser Wavelength	Optical Filter Name	Color
Ch 1	405	SALS	Blue
Ch 2	405	MALS	Blue
Ch 12	405	LALS	Blue
Ch 3	405	BP445-50	Blue
Ch 4	405	BP530-40	Green
Ch 5	405	BP676-36	Red
Ch 6	405	LP750	Dark Red
Ch 7	638	676-36	Red
Ch 8	638	LP750	Dark Red
Ch 9	488	530-40	Green
Ch 10	488	575-30	Orange
Ch 11	488	LP650	Red