



Before starting your measurement, check if you are the first or the last user of this day!

Turn on CytoFLEX > Turn on computer (if you are the first one on this day) > Log in PPMS Tracker log in with your BOKU login > **Log in CytExpert** User > PW: password, start CytExpert software.

Control sheath and waste level

If necessary, fill up sheath fluid (= MilliQ water) and empty the waste during standby mode, the waste tank has to be filled with DanKlorix so that the bottom is covered (located on the right side at the end of the room under the bench). Afterwards: > *Cytometer* > *Prime*. The MilliQ unit and the sink are located in the lab kitchen (room B01-010).

Choose the right injection mode

In the right lower corner, you see the actual injection mode (you need to click on the grey point of the Tracker to see it), if you want to change: > *Cytometer* > *Sample Injection Mode* (semi-automatic for tubes, plate loader for plates, manual is not used at all).

If you change the injection mode, turn off and on again the CytoFLEX and turn around the switcher above the sample probe (plate loader > *P* faces the user, semi-automatic > *P* points to the right side).

Run the start-up program (if you are the first one on this day)

> *Cytometer* > *System Startup Program*


Choose the right plate type, define the three wells with water in your plate as deionized water (DI) wells and set other blue marked wells as empty wells > *Initialize* > *Start* (It takes 10 min).

Run QC (if you are the first one on this day)

> *QC Standardization* > *Start QC Standardization*

Choose the right Lot. Number, take the readily-prepared tube out of the QC beads box in the fridge (if the tube is empty, take 3 drops of the QC bead solution and add 1ml DI) shake well and put 150µl in a well, select plate settings, choose the right plate type, select the appropriate well > *Initialize* > *Start*.





If QC failed because of too few events, close QC with: > *File* > *Close QC standardization*.

> *Open Experiment*, choose *cleaning_plates.xit*. Fill 4 wells with 250µl hypochlorite solution (50ml tube Hypochlorite CLEANING, in the fridge), 4 wells with 250µl DI, 4 wells with 250µl Cleaning Agent and 2 wells with 250µl DI. Select , select the right plate type, select the filled wells as sample wells, select 55sec in stopping rules > *Ok*. Select sample flow rate *Custom* and 240µL/min, start cleaning with auto record, afterwards close the experiment: *Cytometer* > *Close Experiment*, and do the QC again.

If QC passed close QC with > *File* > *Close QC Standardization*.






Run cleaning panel

> *Open Experiment*, choose *cleaning_plates.xit*. Fill 2 wells with 250µl Cleaning Agent and 2 wells with 250µl DI. Select , select the right plate type, and define the filled wells as sample wells. If this is not possible, because these wells were already measured (indicated by either  or , add a new plate with  and define the needed wells on this plate as sample wells). Select 120sec in stopping rules > *Ok*. Select sample flow rate *Fast*, start cleaning with > *Auto Record*, afterwards close the experiment: > *File* > *Close Experiment*.

Creating a new experiment

> *New Experiment*, select your own folder and name the experiment as you wish. Every experiment file has the ending *.xit*. By saving a new experiment, the software creates a folder with the same name as the experiment where your *.fcs* files are automatically saved.

Select  and add plate , select the right plate type and the sample sequence, define the filled wells as sample wells, select the channels and create label names under the channel tab, set compensation by importing previous measured compensation experiments under the compensation tab if needed, select the stopping rules under the stopping rules tab > *Ok*. Create plots and gates as desired with the toolbar, to change an axis name select the axis. Click on the heading area of a plot to select the population/gate, which should be displayed. Select the small grey circle in the right upper corner to display the plot properties (here you can switch between lin and log scale and set the min and max for the axes), if you choose log a slider appears in the left lower corner. By dragging the slider, you can change the lin-log coefficient.

Open the statistics plot , right mouse click on the plot > *Statistics Settings*, and then select the settings and parameters you need.

During run mode, stopping criteria are not working, you have to stop manually. You can change the gain settings and you can overwrite the data.

During record mode, stopping criteria are working and you cannot change your gain settings.

A well measured in record mode cannot be measured again. If you change a *Sample Well* which was already measured to *Empty Well*, the previously acquired data of this well will be permanently deleted.

Open the acquisition settings; mark the first well and start with > *Run* to make your settings. Select the hand button to shift the part of the axis which is displayed. The CytoFLEX is always measuring 7 decades; in the upper section, you have a better resolution. Select the hand button with the G for changing the gain (gain of the fluorescent channels should be higher than 1). Select the threshold tab in the acquisition window or use the threshold button to set the threshold. When you are done with your settings, switch to > *Record*. Afterwards, select the measured well with a right mouse click > *Apply Well Settings To* > Select all sample wells > *Ok*. Start your measurement with > *Auto Record*.



Creating a new experiment from template

> *New Experiment From Template* > *Browse* (first line). Select your own folder and name the experiment as you wish > *Browse* (second line). Select a template (ending *.xitm*).

Select  and add plate , select the right plate type and the sample sequence, define the filled wells as sample wells > *Ok*.

Open the acquisition settings; mark the first well and start with > *Run* to make your settings. When you are done with your settings, switch to > *Record*. Afterwards, select the measured well with a right mouse click > *Apply Well Settings To* > Select all sample wells > *Ok*. Start your measurement with > *Auto Record*.

Creating a compensation experiment

> *New Compensation*. Select your own folder and name the compensation experiment as you want (ending *.xityc*), select the right plate type and sequence, select the channels requiring compensation calculation and the sample type (you need a positive control of each fluorescent channel and a negative control if you have no negative population in your positive controls). Assign the well location by selecting the fluorochrome and setting the appropriate well as *Sample Well* > *Ok*. Measure all automatically created samples > *Auto Record*. Ensure that the gating is appropriate for each sample > *Settings* > *Compensation Calculation*. The compensation matrix appears > *Save as*. Choose your folder and save it (ending of a compensation file *.xityc*).

For saving as template

> *File* > *Save as Template* (save it in your personal folder), every template has the ending *.xitm*.

Transfer your data

YOU ARE ABSOLUTELY NOT ALLOWED TO USE YOUR OWN USB DEVICES IN THE CORE FACILITY OR TAKE USB DEVICES FROM THE CORE FACILITY WITH YOU!

Transfer your data with e.g. BOKU box, Drop box, <https://files.boku.ac.at/>,..

Run cleaning panel (if you are not the last user of the day) See page 2

Fill up sheath fluid and empty the waste (if you are the last user)

> *Standby Mode*. Fill up the sheath fluid (= ultrapure MilliQ water), empty the waste to the sink, put some hypochlorite solution (DanKlorix – located on the right side at the end of the room under the table) to the waste tank, so that the bottom is covered > *Cytometer* > *Prime*. The MilliQ water unit and the sink are located in the lab kitchen (room B01-010).



Run daily clean and shut down the instrument (if you are the last user)

> *Cytometer* > *Daily Clean*. Select the right plate type, set marked wells as empty wells, set 3 wells with cleaning agent as Cleaning Agent well and 5 wells with DI as Deionized water wells > *Start*. After finishing: exit the software with > *File* > *Exit* and switch off the CytoFLEX and shut down the computer.

Booking

All bookings are done via the PPMS booking software. If you are an autonomous user, you do not need a confirmation by CF staff. Just for the first measurement after your training, you will need a confirmation since you are a novice user then (your booked timeslot is red colored until it is confirmed, then it changes to blue)

The timeslot “*Startup*” in the calendar means that Core Facility staff will do the start up of the instrument on that day (depends on availability!), which includes the startup program, QC and the cleaning panel.

Canceling an appointment

If you need to cancel an appointment within 24 hours before start of your instrument time, AND you are the first user of the day AND there is no timeslot with “*Startup*” in PPMS, you absolutely need to contact the next user, since the whole start up procedure takes about 25min. If you are the last user of the day, you absolutely need to contact the preceding user to make sure that the instrument shut down is performed. If you open the timeslot of the other user by left mouse click, you will find the phone number. If **you are not able to contact this person for whatever reason, you absolutely have to perform the start up or shut down yourself.**

Access to the flow cytometry room

As soon as you are an autonomous user, you will also receive access to the flow cytometry room with your BOKU Card during **standard working hours** from **8:00 am to 7:00 pm, Monday to Friday.**

Access to the flow cytometry room outside standard working hours (between 7:00 pm and 8:00 am and on weekends and public holidays)

Before every measurement session outside standard working hours, you need to take a ticket of the Facility Management Portal (FM Helpdesk in order ensure upregulation of the ventilation. This has to be done at least one working days in advance (until 10:00 am).

- Log in on the boku helpdesk
- Choose Request (Anforderungen)
- Choose Air Conditioning on the menu Fault Reports / Repair Work (Lüftung unter Menüpunkt Störungen / Reparatur)



Your personal BOKU Card will open the key safe (located on the right side of the door) 24 hours 7 days a week. In this key safe, we store an emergency device together with an access card, which opens the flow cytometry door. If you are working outside the standard working hours, you absolutely have to make use of our emergency device, which helps to ensure your personal safety when working alone in the lab. More information on the usage of this device is provided in a separate document (*“How to use the emergency device”*) which can be found in the flow cytometry room.

Outside standard working hours: Keep the emergency device on your body at any time during usage of the flow cytometry room. Never remove the BOKU Card from the emergency device.

Don't forget to turn off the emergency device, when you are done before putting it back into the key safe.