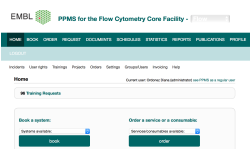


Getting started at the FCCF

Welcome to
the FCCF



CREATING A PPMS
ACCOUNT



ACCESS TO THE
ANALYSERS



TRAINING SESSION

We are happy to see you and help you with all your flow related needs including; experimental design, sample acquisition, data analysis and cell sorting. In this document you will find indications to fully access all the services offered by the flow cytometry core facility!. If you require additional information write us an email to: fccf-team@embl.de or pass by the facility, we are located in the annex building (Operon) at room V101-V104.

Creating a ppms account

In order to book our services you need to create an account in the ppms booking system.

Please use the link <https://hd-ppms01.embl.de/areq/?pf=3> to request an account. As we are connected to the EMBL universal identification server, use your EMBL login details. When the system asks you to write in your login with the @embl.de at the end, please only put your actual regular EMBL login into this field and not your email address. After that, just use your regular EMBL login password.

If it works, you will be prompted to a small page where you can fill in details and once that is done, we will receive an account creation request through the system. Your request will need to be accepted by the facility team members, please expect a bit of waiting time until we have your account online.

Sort requests can be found in the booking system under the “Request” tab. You will need to fill a request format with information related to your samples and preferential dates for your slot. The sorting slot will be then confirmed by members of the facility after analysing the needs of your experiment (summarised in the request format) and the availability and configuration of the instruments. The schedules of the sorters are available only for visualisation of the occupancy **but not for reservation by the users**.

Reservations of the analysers and FlowJo dongles could be done in the “Schedule” tab. Note that only trained users are authorised to book the analysers. Have a look around the

software and please let us know if you experience any problems. We are available to help you creating your account if needed.

Access to the analysers

To use our analysers you must complete a training session in which you will learn basic concepts about flow cytometry and instrument configuration and you will also have the opportunity to interact with the acquisition software. To request one, use the "Request" tab in the PPMS booking system or write us an email to: fccf-team@embl.de

After completing the training you will gain access to the analysers' room (V104) using your badge and **you will be able to reserve the analysers** using the "Book" tab in the ppms booking system. As we do not expect you to be totally independent after the training session, you could ask for assistance when making your reservation, please ask in advance to make sure someone from the facility is available for helping you.

Training session

The session takes around 3 hours and include:

Brief description about flow cytometry principles	40 min
Cytometer Operation (BD Fortessa/Accuri C6)	2h min.
Data Analysis in Diva/Accuri C6 Software	20 min

You will learn:

General concepts and principles behind flow cytometry: Fluidics, Optics and Electronics
How to operate the cytometer: Sample acquisition and experiment set-up (threshold, gates for acquisition, doublet and dead cells exclusion) and
Data analysis: Gate definition and basic stats.

We need samples for the training!!

Bring your own samples with the regular staining you perform or expressing the fluorescence protein of your interest, like this we could adjust and record settings for your future experiments.

Samples to prepare:

1. Negative control: Unstained cells/ Untransfected cells (no fluorescence signal)
 2. Positive control for every fluorescence signal: Stained cells/Transfected cells
- If more than one fluorochrome or fluorescence protein will be evaluated, prepare cells expressing only one fluorescence signal at once.
3. Samples to analyse: Please bring few samples (no more than 5), like this we could dedicated more time playing with the acquisition software.

* If your staining includes primary + secondary antibody, prepare a sample stained exclusively with your secondary antibody, in order to evaluated unspecific binding of the antibody.

If you need additional information regarding sample preparation or staining protocol, feel free to contact the facility.