

FACS Service – Group Detmar, ETH Höggerberg

We are providing a high speed, high quality cell sorting service, using a BD FACSAria Cell Sorter. Equipped with 2 lasers (488nm & 633 nm), the sorter is capable of providing 9-parameter, 7-color sorting/analysis.

Location

Group Detmar
ETH Höggerberg
HCI H394.1

Operator

Cornelius Fischer – Technician
Phone 044 633 64 30 (ETH intern 36430)
Mail cornelius.fischer@pharma.ethz.ch

General information

All sorts are charged with **CHF 220/hour** for external customers.

Members of the IPW Institute will be charged with **CHF 120/hour**.

We charge for sort time and setup time (FSC/SSC & compensation).

Billing time is automatically charged for the first 30min and afterwards in 15min steps.

The time booked is the time charged (i.e. you cannot book the FACS sorter for 4 hours for a sort of only 1 hour).

Scheduling

General service days are **Wednesday to Friday**. Please be aware of the following reservation rules:

- Please book your slots about 2 weeks in advance by email. Otherwise we cannot guarantee for free slots
- Reservations will be confirmed by email
- Cancellation policy: Sorts have to be cancelled **two working days** before your sort, or you will be charged for the reserved time. E.g. Tuesday sorts have to be cancelled by Friday afternoon, or Wednesday sorts have to be cancelled on Monday before noon.
- **Please be on time**, especially for the late afternoon sorts. If you are running more than 15min late, please contact the operator as soon as you know that you will be late. If you are late, there is no guarantee that additional time will be available after your reserved slot.

Sorting & Sample preparation

We provide a full sorting service. That means, all sorts will be done by our operator. You are not allowed to use the sorter by yourself, but you also do not have to wait at the sorter. Feel free to go back to your lab, after setting up the machine and come back when your cells are sorted.

Sort Setup (pressure)	Nozzle size	Application
High (70psi)	70 μm	High-speed sorting, robust cells
Medium (35psi)	70 μm	Sorting of fragile cells, sensitive cells
Low (20psi) <i>standard</i>	100 μm	Single-cell, large or fragile cells, adherent cell lines

Samples MUST be filtered right before the sort through a 40 μm filter

→ such as BD cell strainer (#352235)

The FACSAria uses a nozzle tip to generate a stream of sheath fluid. This nozzle has an

orifice (70 μm or 100 μm) that when clogged results in the critical failure of the stream and most likely the sort as well (sample collection tubes can become compromised with sheath carrying non-target particles/cells as a result of the clog). As procedures and reagents used during sample preparation can induce clumping, such as centrifugation and high serum concentrations, filtering of samples should be the last step before they are sorted.

Minimum Sample Volume: 0.5 ml (500 μl)

The FACSAria can accommodate 1 ml, 5 ml, and 15 ml sample tubes. Samples should ideally be resuspended in a standard PBS based FACS buffer with minimal serum (i.e. PBS + 1-2% serum).

Higher serum concentration or sorts directly from media are possible, but not recommended due to a higher risk of cell clumping during the sort. Please contact the operator if you need a sort with higher serum concentration.

Sample Concentration

Sort Setup	Optimal Concentration (total cells/ml)
High	40 x 10 ⁶
Medium	20 x 10 ⁶
Low	10 x 10 ⁶

The system has several precision modes. The most commonly used mode is "purity." In "purity" mode the system will always maintain purity at the cost of yield. Samples at high concentration, those above the optimal, will take less time to sort but will sort with a lower efficiency relative to those at optimal concentration. Samples at low concentration, those below the optimal, will take longer to sort but will sort with a higher efficiency relative to those at optimal. Please keep this in consideration when designing your sort experiment and scheduling time.

Collection Devices

- 1-Way sorting (single cell) into 6well, 24well, 96well & 384well plates
- 2-Way sorting into 1 ml, 5 ml, and 15 ml collection devices
- 4-Way sorting into 1 ml and 5 ml collection devices

The FACSAria is equipped with a re-circulating water bath that allows specially designed 15 ml (2-way) and 5 ml (2-way/4-way) collection devices to be maintained at a set temperature point. The default set point is 4°C but can be modified to suit particular needs.

Collection Tubes

Media in collection tubes should contain at least 10% serum. The appropriate volume of media for 5 ml or 15 ml collection tubes is 1 ml or 4 ml per tube, respectively.

It is important to pre-coat collection tubes with media + serum to increase post-sort viability and recovery. This involves coating the entire inner surface of the collection tube. Sorted cells that hit bare plastic have the potential to stick and dry out.

To aid in the recovery of a sort population of low percentage, a high volume of media in a conically shaped collection tube is recommended (13 ml in a 15 ml conical tube).

Sheath Fluid

Standard Sheath Fluid is 1x PBS (NaCl, KCl, Na₂HPO₄·7H₂O, KH₂PO₄ in water) with pH 7.4

Color Setup

The sorter is equipped with two lasers (488nm & 633nm) for use in multicolor sortings with maximum 7 colors.

PMT	488nm					
	1	2	3	4	5	6
Long Pass (LP)	735	655	595	556	502	none
Filter	780/60	695/40	610/20	576/26	530/30	488/10
Typical Fluorochrome (Em-Max)	PE-Cy7 (785nm)	PerCP-Cy5.5 (695nm) 7AAD (660nm)	PE-Texas Red (615nm)	PE (578nm)	FITC (519nm)	none (SSC)

PMT	633nm	
	1	2
Long Pass (LP)	735	none
Filter	780/60	660/20
Typical Fluorochrome (Em-Max)	APC-Cy7 (785nm)	APC (660nm)

To plan your experiments and virtually test your fluorescence combinations, check out the BD spectra view website:

<http://www.bdbiosciences.com/spectra>

If you have more questions or like to reserve an appointment, please contact *Cornelius Fischer*.