QMRI Flow Cytometry
Cell Sorting facility

Sorting Guidelines

The QMRI Flow Cytometry and Cell Sorting Facility offers an operator-based sorting service to its researchers. These guidelines are to provide researchers who wish to sort samples with an outline of how to arrange a sort and what information is required by facility staff to optimise your sort and any biosafety issues that need considered. There are also sections detailing how to prepare for your sort and what to bring to the facility

Facility e-mail:	qmriflow@ed.ac.uk

This e-mail (not individual addresses) should be used for correspondence relating to sorting bookings.

Flow Lab Core Hours:	Monday to Friday 9am-5pm
Sorting Core Hours:	Monday 11am-5pm, Tuesday to Thursday 9.30am-5.30pm, Friday 9.30am-4pm
Instrument Maintenance:	Monday 9-11am and Friday 4-5pm

Booking

- All internal users of this service must have a user profile on the online booking system and have submitted a completed application form.
- External user sorts will generally be booked directly with staff.
- Cell sorters are highly subscribed instruments. Sorts with 8 days advance notice and during core hours can be booked by registered users using the online request form via PPMS. If you are unable to sort at a desired time, please contact staff as we will do our best to accommodate you. Alternatively, you can request a notification of cancellation through the online booking system and contact staff if a slot becomes available.
- The request will be assessed by facility staff and the user will receive a confirmation notification by e-mail. Please do not setup experiments until the sort has been confirmed. Sorts are allocated to specific operators; however, this is subject to change. Always use the facility e-mail, or online booking form, for any contact with regard to the sort.
- The online request form is necessary to allow staff to optimise the sorting time. Failure to complete this properly may result in delays.
- Staff must be notified (via the online sort form on the booked sort) immediately regarding any changes to the agreed sort request. Cancellations must be made by the user using the online booking system to allow correct calculations of charges.
- Sorts required or extending outwith core sorting hours (see above) must be arranged directly with staff using the facility e-mail. **Do not** use individual e-mails for this purpose.
- The facility maintains and QCs the instruments on a daily basis. If an instrument breaks down, users will be notified immediately via e-mail. If an instrument breaks down, no charges will be raised for any sorts that cannot be done though we will always strive to allocate slots on another instrument, if available.

Biosafety

- All sorts require a completed QMRI Flow Lab Biosafety form and a copy of the relevant Risk Assessment form.
- All sorts using referenced human blood must be recorded in the relevant facility folder.

Sample information

It is important to the quality & efficiency of the sort that you provide us with as much information as possible and complete the online form to reflect this.

1. **Total cell concentration**
   Researchers are expected to know the cell concentration of the samples for sorting. Remember that if you count cells before staining then the cell number will be reduced at the end of your protocol. Please
   note that total particle number includes non-lysed RBCs and debris, as these have an impact on the sort quality and time. We will recommend that lysis be repeated if the sample is found to be red in colour as the sort quality will be diminished.
2. **Straining**
   Depending on cell type, we will recommend that sort samples should be strained (35 or 40 µm) immediately prior to the sort. This reduces the potential for clogging and decreases the risk of aerosolization.
3. **Nozzle size**
   Nozzle size is important. In general, the maximum cell size, within the sample should be less than $1/7^{th}$ of the nozzle size. We routinely use a 70 µm nozzle for primary cells such as monocytes and neutrophils and a 100µm nozzle for cell lines, macrophages, DCs and tissue digests. Plate sorts should not be booked with the 70 µm nozzle. Larger cell sorts should be booked on the Influx which has both 140 µm and 200 µm nozzles. Please not that this requires extra time for stabilisation. Nozzle size should be selected during the booking process. If you are unsure of which nozzle size to use, please arrange the booking directly with staff.
4. **Pressure**
   The nozzle size determines the pressure. Reducing the pressure minimises the stress on the cells and improves post sort viability. This pressure is set to 70 psi for the 70 µm nozzle and 20 psi for the 100 µm nozzle. Pressure settings on the Influx can be changed more easily but we tend to use 7 psi for the 140 µm nozzle and <5 psi for the 200 µm. Pressure determines the frequency of droplet formation and thus the maximum event rates. Please contact staff to discuss this further.
5. **Cell Concentration**
   Samples requiring the 70 µm nozzle should be at a concentration of at least 20-40 $x10^6$ cells/ml and for the 100 µm nozzle at a concentration of 5-10 $x10^6$ cells /ml. Concentrations much lower than this may result in the operator increasing the sample flow rate (to allow the sample to be processed in time) which in turn will impact on the resolution of populations. If you are unsure of cell number, we recommend that you bring us the samples more concentrated and we can

dilute as required. Concentrations may need to be reduced when using 4 way or single cell sort modes

6. **Timings**
   It takes approximately 25-30 minutes to sort 1 ml sample at the above concentrations, when running a 2 way purity sort. Samples, which are too concentrated will not sort well as they are prone to pressure fluctuations and those too dilute will take longer than necessary. For small volume samples, which are known to be less than the recommended concentration, please discuss with staff. Staff will be able to assess the likely sort time at the start of a sort. This may require a compromise if the sort slot is too short. Please also remember that these instruments sort cell populations and cannot recover more cells than are present in the initial sample.
7. **Sort Medium**
   Cells for sorting should be re-suspended in medium containing less than 2 % serum to prevent build-up of protein in the tubing. Using no serum is an option but may lead to loss of cells due to stickiness to the tube. We recommend that you bring some spare sort medium in case we need to dilute your sample.

Collection

- Well labelled collection tubes containing appropriate medium should be provided to the operator at the start of the sort. Operators should not be expected to do this as we have no immediate access to tissue culture biosafety cabinets and therefore cannot ensure sterility. Users may be asked to stay if there is no clarity to the samples for sorting.
- When using plates, please provide an exact spare plate for setup of the ACDU (Automated Cell Deposition Unit). When using 384 well plates, we have found slight differences in the grid pattern between plate manufacturers.
- We recommend that the sorting medium contains at least 10 % serum. Staff tend to vortex these tubes before starting the sort as this will coat the tube surface and encourage the sorted cells to slide slowly in to the medium, improving post-sort survival. It also prevents the sorted drops and the cells contained within from sticking to the tubes and reducing post sort cell counts. Please note that when the cells are sorted they are surrounded by the sort medium and contain a volume – this volume will dilute your sorting medium and thus the serum content.
- Sorting modes determine the purity/yield of the sorted populations. Please discuss your sample requirements with your operator.
- Collection tubes should be chosen optimally for the cell number which is likely to be collected. Too large a vessel will result in loss of cells and too small will require frequent tube changes or a set limit.
- Spare prepared collection tubes should also be provided in case they are required.
- Staff must be made aware of the collection medium used and a data sheet must be provided for new reagents.

- This table is a rough guide to help assess expected volumes when using the main nozzles and with a sort mode of 16-16-0.

	70 µm	**100 µm**
1 cells	0.0016 µl	0.005 µl
1,000 cells	1.6 µl	5 µl
10,000 cells	16 µl	50 µl
100,000 cells	160 µl	500 µl
1,000,000 cells	1.6 ml	5 ml

Post-Sort

Please inform staff asap if you have any issues with your sorted samples. This will allow us to investigate the matter further, if necessary.