

Instructions for Sort Request Form:

We appreciate it if you try to fit your request onto a single page when possible. Email completed forms to parcfacs@partners.org.

Please include all user information—your full name, your email, a phone number in case I need to call you during the sort, department (and acronym if applicable), PI on the grant that you will be using (FULL NAME, not just last name), and grant number (do NOT just say “Same as other person”—find out what the number is).

PIBC number: Please see the “sorting guidelines” document on our website for full details. Provide the PIBC Protocol number of the project that is being sorted. This is only required for unfixed human or primate samples, or infectious samples. If your sample is not pathogenic and not human (e.g. normal mouse), then just put “N/A”.

Please provide a first and second choice for date of sort, and time window that works for you (after 1pm, any time between 10 and 2 pm, etc) in case the first choice is already booked. Include an earliest start time as mornings are usually more open than afternoons. ****If you save a copy of it to re-send me, make sure you update your information—the dates you are requesting and the fund number if that has changed.****

Sample information:

Species: (human, mouse, hamster, etc)

Cell type: indicate primary cell or cell line, include tissue of origin.

Biosafety Level: If you don't know, check with your safety officer BEFORE sorting!

BL1 are samples that are totally non-infectious to humans, and contain no human materials. BL2 are any samples that are of Human or non-human primate in origin, such as cell lines. BL2+ are infectious samples such as HIV+ patient samples, and any primary cultures of human cells (blood draws, etc).

Hazards, Infectious agents: this includes any KNOWN diseases a patient sample may carry. Please also indicate any transfection agents used on the sample. User must also provide the symptoms/signs of infection in the event of an accidental exposure, either on the sort request form the first time, or as a separate document to be kept on file in the core. If no known infectious agent is present, then please enter “uninfected cell line” or “Healthy Donor” or some other indication of sample status.

Number of samples to sort: how many different samples to be sorted (do not include single stained controls in this number). If additional tubes are being acquired for data, please include here as well.

Cell number per sample: estimate how many cells are in each sample tube(s).

% of cells that fit sort criteria: what % of TOTAL EVENTS fit the parameters you want to sort by (if sample is 10% GFP+ and you want the top 10% of GFP, then 1% fit the sort criteria)

Total cell # you wish to collect: the number of cells where I can stop sorting, you don't want more than that. “Entire sample” is also acceptable to enter here.

Minimum # of cells you can use: what is the smallest number of cells that are useful to you. If I can only get 10k and you can't do your experiment with less than 100k, then it doesn't make sense to run the sort—cells will have to be expanded, or more animals included next time.

Fluorochromes used and gating scheme: list the fluorochromes in your experiment and what they are labeling, and then identify the gates used to identify your target population.

For example:

FITC-Lin

PE-cKit

APC-Sca1

Sort FITC low, PE+, APC+

Printout of analyzed sample would be helpful for proper gating.

If there are not clearly defined + and – populations, please specify your gating preferences (above autofluorescence, top 10%, e.g. Treg cells are often identified as the top 30% of CD25 stain)

Instrument Info:

Format: Type of sorting, either bulk (into tube) or plate (e.g. cloning into 96 well plate)

Purpose of sort: indicate whether you are going to collect RNA, culture the sample, etc.

Special Instructions: include any special instructions here such as:

- for RNA extractions sorting into lysis buffer, sample should be mixed every 10 minutes
- Pressure: stream pressure can be adjusted. The usual setup is 70 psi, if you have delicate/fragile cells, lower pressure may improve post-sort viability. 96 well plates will be run at lower pressures. Lower pressure bulk sorts will take longer as the maximum event rate is lower.
- Nozzle size: Ideally, nozzle should be 3-4 times larger than the cells being sorted. Larger cells may cause fanning/spraying with the smaller nozzle, and may benefit from using the larger nozzle, but the larger nozzle size has a lower maximum event rate (20 million per hour instead of 60 million per hour).