## Flow Cytometry Core (FCC): FACS Sort Request Form

## A Chemical Biology of Infectious Disease (CBID) COBRE Core Laboratory

**KU Flow Cytometry Core**

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**This core facility is registered with the University of Kansas’ Department of Environment, Health & Safety (KU EHS)** **as a BSL-2 facility and has permission to perform cell sorts on non-fixed virus-infected specimens.**

All specimens must be transported in accordance with KU EHS regulations. Specimens arriving from inside the Haworth building must be in a leak-proof primary receptacle (tube) in a sealed leak-proof secondary container. Specimens from outside of Haworth require triple layer packaging: a leak-proof primary receptacle (tube), a leak-proof secondary packaging, and an outer packaging box/cooler.

**Unfixed** specimens will be considered as potential biohazards and processed under BSL-2 requirements.

For all **fixed** specimens, appropriate and reliable methods must be used to inactivate potentially biohazardous agents (e.g. freshly prepared formalin solution: 1% for 30 min). These procedures must be performed CAREFULLY; otherwise, samples that are considered to be inactivated, but in fact are not, can pose a serious health risk to laboratory staff.

**Rates**

**$50 set-up fee + hourly rate below:**

KU Internal External Academic External Market

$22/h $34/h $66/h BD FACSymphony S6

$20/h $31/h $60/h BD FACSAria Fusion

$40/h $61/h $120/h Specialized service, labor

**Policies**

The FCC must be notified of any cancellations by 4:30 PM on the day prior to the sort date

or the investigator may be billed for the time he or she scheduled.

**The sort request form must be given to the flow facility a minimum of 48 hours prior to the sort. The investigator must be accessible during the sort.**

Cells should be filtered through a filter or cell strainer to remove cell aggregates. The following options for cell strainers fit into standard 15 mL and 50 mL conical tubes:

* 30μm, for 15mL tubes, Miltenyi Biotec MACS Pre-Separation Filter (cat. No. 130-041-407), $245/case of 50.
* 40 μm, for 15mL tubes, Fisher Scientific 07-001-106 EASYstrainer Cell Strainer, Greiner 542140, Green Sterile, 50/case filters, $115/case of 50.
* 40μm, for 50mL tubes, Fisher Scientific 07-000-222 EASYstrainer Cell Strainer, Greiner 542040, Green Sterile, 50/case filters, $115/case of 50.

Ideally, cells to be sorted should be suspended at a concentration of **5-10 million cells/mL** in Ca and Mg free 1X PBS only. Lower cell concentrations are sometimes unavoidable but the concentration should not exceed 10 million cells/mL. Cells should be suspended in BD Falcon 5 mL round-bottom polystyrene tubes (cat. No. 352054) with no less than 500uL and no more than 3mL of sample per tube.

Bring appropriate control samples: Negative control, Positive control (if needed), Cells or antibody- capture beads stained as single color controls with each antibody used in your experiment.

If sorting into plates rather than tubes please bring enough plates for collection, **plus one more** for aligning the sort stream.

**SORT REQUEST FORM**

**Investigator: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ PI: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Email: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Phone: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Sort Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Time Preferred: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Number of Samples: \_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**COMS / IBC registration number and title (for recombinant material and infectious agents):**

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**IRB protocol number (for human specimens, if applicable): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**IACUC protocol number (for animal specimens): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Biosafety Level of this experiment (listed in COMS/IBC registration): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Grant code / PO # (if applicable): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**1. List Type of sample: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**a. Eukaryotic cells (check one)**

**i.  Human  Rodent**

 **Transgenic/grafted (list donor & host species): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

 ** Other: \_\_\_\_\_\_\_\_\_\_\_**

 **ii. Description:  Cultured cells  Freshly isolated primary cells  Cell line (ATCC#)\_\_\_\_\_\_\_\_\_\_\_\_**

** Blood  Primary cells  Other: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**b. Bacterial cells:  ATCC#\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**2. Will the sample be fixed before delivery to the sorting facility?  No  Yes**

 **If Yes: list the fixation agent, it’s concentration and the exposure time: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**3. Have the samples been exposed to, or contain any infectious agents?**

* **No**
* **Yes (Describe): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**4. If samples are of human origin, have they been screened?**

* **No**
* **Yes – Describe: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**5. Does the sample contain any recombinant genetic material? Have the samples been genetically modified?**

* **No**
* **Yes – Describe vector (adenovirus, retrovirus, lentivirus, replication competent/defective, tropism, oncogenes), recombinant construct, gene inserts (HIV/SIV inserts etc), ecotropic, amphotropic, days after infection, etc:**

 **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**6. Are there any hazardous agents in your sample or collection media that the sort operator should be aware of?**

* **No**
* **Yes – Describe: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

# Sort Information

1. **Description of your experiment (in relation to flow cytometry):**
2. **Number of cells per tube(s): Approximate % of sort population in sample(s): Number of target cells to be collected:**
3. **Are the cells adherent?**
* No
* Yes (refer to protocol for sorting live adherent cells: trypsin should not be used as it reduces expression of cell surface markers.)
1. **List all fluorochromes and markers used, including viability dye, or fluorophore panel with associated markers (if applicable). Laser configurations for the BD FACSAria Fusion and the BD FACSymphony S6 are on the following pages. E.g., matching laser to antigen to available channels / exemplary dyes; different configurations on each instrument.**
2. **Please provide the FCC sort operator with 2x the expected volume of collection media or sterile filtered FBS.**
3. **Provide details of gating strategy. If possible/necessary, please draw or copy-paste the gating strategy you would like the FCC sort operator to use for sorting your sample, including:**
* **Make sure your gating hierarchy and sort gates are clearly shown in your design.**
* **Indicate the populations to be collected.**
* **Provide details of expected % for each population, if known.**
* **Number of cells to be collected per population: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**
* **Controls provided:**

 **Unstained control**

 **Individually labelled fluorophores / single stains**

 **FMO: Fluorescence Minus One controls**

 **+/- Positive/negative, activated/inactivated, etc**

 **Compensation controls**

** Isotype control (an antibody selected as a negative control for flow cytometry experiments, and is used to detect non-specific binding)**

 **Other controls: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

* **Type of sort:  Bulk  Single Sort**
* **Collection vessel:  15mL tube  FACS tube  1.5mL microtube  slides (std / frosted)  Multiwell plate:  6-well  12-well  24-well  48-well  96-well  384-well**
* **Temperature of samples during sorting:  RT  4°C  37°C  other \_\_\_\_\_\_\_**
* **Temperature of collection tubes/plates:  RT  4°C  37°C  other \_\_\_\_\_\_\_**
* **Sterile sort?  Yes  No**
* **Purity check required?  Yes  No**
* **Cell Diameter: \_\_\_\_\_\_\_\_\_\_\_\_\_µm**
* **Collection media/FBS/PBS/other (see guidelines): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**
* **Other cell details, e.g. cells are:  Robust  Fragile  \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**BD FACSymphony S6 – five laser**

**Panel / Fluorochromes to be used and the gating strategy:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Laser** | **Band** | **Fluorochrome** | **Target** | **Notes/ Gating** |
| 355 nm UV Laser | BUV396 (379/28) |   |   |   |
| 355 nm UV Laser | BUV496 (450LP; 515/30) |   |   |   |
| 355 nm UV Laser | BUV563 (550LP; 586/15) |   |   |   |
| 355 nm UV Laser | BUV661 (635LP; 670/30) |   |   |   |
| 355 nm UV Laser | BUV737 (690LP; 740/35) |   |   |   |
| 355 nm UV Laser | BUV805 (770LP; 820/60) |   |   |   |
| 405 nm Violet Laser | BV421 (410LP; 431/28) |   |   |   |
| 405 nm Violet Laser | BV480 (450LP; 470/14) |   |   |   |
| 405 nm Violet Laser | BV605 (600LP; 610/20) |   |   |   |
| 405 nm Violet Laser | BV650 (635LP; 670/30) |   |   |   |
| 405 nm Violet Laser | BV711 (690LP; 710/50) |   |   |   |
| 405 nm Violet Laser | BV786 (750LP; 780/60) |   |   |   |
| 488 nm Blue Laser | BB515 (505LP; 515/20) |   |   |   |
| 488 nm Blue Laser | BB630 (600LP; 610/20) |   |   |   |
| 488 nm Blue Laser | BB700 (690LP; 710/50) |   |   |   |
| 488 nm Blue Laser | BB790 (770LP; 780/60) |   |   |   |
| 561 nm Yellow-Green Laser | PE (570LP; 586/15) |   |   |   |
| 561 nm Yellow-Green Laser | PE CF594/PE-Texas Red (600LP; 610/20) |   |   |   |
| 561 nm Yellow-Green Laser | PE Cy5 (650LP; 670/30) |   |   |   |
| 561 nm Yellow-Green Laser | PE Cy7 (750LP; 780/60) |   |   |   |
| 637 nm Red Laser | APC (650LP; 670/30) |   |   |   |
| 637 nm Red Laser | Alexa 700 (690LP; 710/50) |   |   |   |
| 637 nm Red Laser | APC H7 (750LP; 780/60) |   |   |   |

#

# BD FACSymphony S6 Configuration

* Sort 6 populations simultaneously
* 355 nm UV Laser
* 405 nm Violet Laser
* 488 nm Blue Laser
* 561 nm Yellow-Green Laser
* 637 nm Red Laser
* Temperature controlled sorting
* 23-color sorter
* Includes BSC

# BD FACSAria Fusion Configuration:

* Sort 4 populations simultaneously
* 405 nm Violet Laser
* 488 nm Blue Laser
* 561 nm Yellow-Green Laser
* 633 nm Red Laser
* Temperature controlled sorting
* 12-color sorter

|  |  |  |  |
| --- | --- | --- | --- |
| **Laser** | **Detector** | **1o****Fluorochrome** | **Band Pass****Filter** |
| 405 nm 100 mWViolet Laser | A | BD Horizon Brillian Violet | 421, V450 |
| B | BD Horizon Brillian Violet | VPD450 |
| C | Pacific Blue, DAPI | 450/40 |
| D | Violet | 510 or V500 |
| E | AmCyan | 525/50, 505LP |
| F | BD Horizon Brillian Violet | 605 (610/20, 595 LP) |
| G |  |  |
| H |  |  |
|  |  |
| 488 nm 50 mWBlue Laser | A | FSC |  |
| B | PerCP | 675/20 |
| C | PerCP-Cy5.5 | 695/40 |
| D | FITC/Alexa488 | 530/30, 502LP |
| E | SSC | 488/10 |
|  |  |
| 532 nm 150 mWYellow-Green Laser | A | PE/DsRed | 582/15 |
| B | PE-Texas Red | 610/120, 600LP |
| C | mCherry | 610/120, 600LP |
| D | PI | 610/120, 600LP |
| E | PE-Cy5 | 670/14 |
| F | PE-Cy5.5 | 710/50, 655LP |
| G |  |  |
| H |  |  |
|  |  |
| 628 nm 200 mWRed Laser | A | APC-Cy7 | 780/60, 735LP |
| B | Alexa 700 | 730/45 |
| C | APC | 660/20 |

# Cell populations to sort (collect) from the sample (up to 6 sub populations from each sample for Symphony, up to 4 for Aria Fusion):

# BD FACSymphony S6 sorter: Sort 6 populations simultaneously

# BD FACSAria Fusion sorter: Sort 4 populations simultaneously

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| No | Population ID | % of total | # To collect | Theoretical # needed | Markers/Phenotype |
| 1 |   |   |   |   |   |
| 2 |   |   |   |   |   |
| 3 |   |   |   |   |   |
| 4 |   |   |   |   |   |
| 5 |   |   |   |   |   |
| 6 |   |   |   |   |   |

* Calculate theoretical # and recommended # of cells you need to start with: \*Post staining and post filtering cell counts are the most accurate.

Recommended starting# (2x theoretical #) = \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

* Calculate the length of time for each sample: flow rate for medium sort is 6,000/sec, slow sort is 2,000/sec.

Time (Starting number / Flow rate per sample) = \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

* Sample concentration (Recommended: 5 to 25 million cells/ml): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

If possible, please provide your:

* Sample preparation protocol
* Staining protocol

**Treating Adherent Cells for Live Sorting**

* 1. Decant culture media off adherent cells. Add **wash buffer** to cells in a volume equal to the culture media volume: Wash Buffer: **Ca and Mg free** 1X PBS with 10% FBS. Swirl flask. Incubate 10 minutes at 37˚C. Swirl Flask. Decant off wash buffer.
	2. After the final decanting step add **EDTA buffer** at a volume equal to one-tenth of the initial culture media volume: EDTA buffer: 2mM EDTA solution in **Ca and Mg free** 1X PBS with 10% FBS. Swirl flask. Incubate 10 minutes at 37˚C. Swirl Flask. Cells should now be in suspension.
	3. Spin cells down and re-suspend in **Sorting buffer** at a concentration of 10 million cells per mL: Sorting buffer: **Ca and Mg free** 1X PBS, 1mM EDTA, 25mM HEPES pH 7.0 and ***one*** percent FBS : 2uM filter sterilize and store at 4˚C
	4. If sorting into 96-well plates, add 100uL of Sorting Buffer with **10%** FBS to every well. Increasing the FBS amount in the receiving buffer helps maintain cell viability.

**Notes**:

Divalent cations enable the cells to adhere; eliminating them in the buffering solution and adding a calcium chelator (EDTA) allows the cells to be placed in suspension.

Culture media is not an ideal sort buffer for several reasons:

* pH regulation fails under normal atmospheric conditions.
* The calcium chloride in most culture media is not compatible with the phosphate component of the instrument sheath leading to precipitation of calcium phosphate crystals.
* Phenol Red, included in many culture medias, contributes to background fluorescence.

The sorting buffer should have minimal FBS/BSA/FCS – the addition of protein to a sort buffer increases the amount of distorting light scatter thus decreasing the sensitivity of the forward and side scatter signals.

Cells under go increased pressure prior to and during a sort; the partial pressure of CO2 also increases and will reduce the pH of the solution unless it is adequately buffered. HEPES buffer has an optimal buffering capacity at physiological pH. A final concentration of HEPES in a sort buffer with a neutral pH minimizes the acidification of the sample while under pressure.