BIDMC – Center for Virology and Vaccine Research (CVVR) - CELL SORTING FACILITY

Location: CVVR
Center for Life Science (CLS), 10th floor, room 1032B
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Boston, MA 02115

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This cell sorting facility is registered with Harvard COMS as a BL2+ facility and has permission to perform cell sorts on non-fixed AIDS/SIV/SHIV virus-infected specimens. However, unfixed specimens known to contain BL3 or BL4 agents or from individuals with acute TB cannot be accepted. In addition, samples from any acutely ill individual without diagnosis cannot be accepted.

All specimens arriving from outside of the CLS building must be transported in accordance with DOT/IATA regulations. In general, this entails triple layer packaging (a leak-proof primary receptacle, a leak-proof secondary packaging and sealed outer packaging box/cooler). Specimens arriving from inside the CLS building must be in a leak-proof primary receptacle in a sealed leak-proof secondary container.

Unfixed specimens will be considered as potential biohazards and processed under BL-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

$90 per hour + $50 set-up fee

GENERAL RULES

The sorting facility must be **notified of any cancellations by 5PM 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 Miltenyi Biotec MACS Pre-Separation Filter (*cat. No. 130-041-407*). 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 Desired:** _____ **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): _____

1. **List Type of sample:** _____
   a. Eukaryotic cells (check one)
      i. Human ☐ Non-Human Primate ☐ Rodent ☐
      Transgenic/grafted (list donor & host species): ☐_____
      Other: ☐_____
      ii. Description: Freshly isolated primary cells☐cultured cells☐cell line (ATCC#)☐_____

   b. Prokaryotic cells (check one): Bacteria ☐ Fungi ☐ Parasite ☐
      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 any infectious agents?**
   ○No
   ◉Yes (Describe):
   _____

4. **Unfixed samples infected with BL3 or BL4 agents and/or individuals with active tuberculosis, and/or from acutely ill patients without diagnosis cannot be processed in the flow cytometry cell sorting facility.**
   a. Excluding HIV viruses, are there any evidence for active tuberculosis and/or any other active infection with BL3 or BL4 agents?
      ○No
      ◉Yes (Unfixed samples from this individual can not be accepted.)
   b. Is the individual currently acutely ill without diagnosis?
      ○No
      ◉Yes (Unfixed samples from this individual can not be accepted.)

5. **Does the sample contain any recombinant genetic material?**
   ○No
   ◉Yes – Describe vector (adenovirus, retrovirus, lentivirus etc) & gene inserts (HIV/SIV inserts etc):
   _____
   _____

I understand that all persons working with biological materials must know the potential biohazards associated with their work. I have provided accurate information on the origin of the cells to be sorted. I have listed the appropriate biosafety status of my sample(s) and understand that misclassification increases the risk to the staff of the Cell Sorting Facility and that action may be pursued for intentional misrepresentation.

Date: _____ Signature: _____

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 (see attached protocol for sorting live adherent cells – trypsin should not be used as it reduces expression of cell surface markers.)

4. Fluorophore panel with associated markers (if applicable):

_____

_____

_____

5. The Cell Sorting Facility will supply sterile filtered FBS for cell collection. If you require any other collection media you must provide the Cell Sorting Facility with 2x the expected volume of collection media.

6. Please draw the gating strategy you would like the Cell Sorting Facility to use for sorting your sample. Make sure your gating hierarchy and sort gates are clearly shown in your drawings.

FACSAria Configuration

Laser	Detector	1° Fluorochrome	Band Pass Filter
405 nm 100 mW	A	QD800	780/60
	B	QD705	710/50
	C	QD655	660/20
	D	QD605	605/40
	E	QD585	585/42
	F	QD565	560/40
	G	AMCyan	515/20
	H	Pacific Blue	450/50
488 nm 50 mW	A	PerCP-Cy5.5	710/50
	B	FITC	515/20
	C	SSC	488/10
532 nm 150 mW	A	PE-Cy7	780/60
	B	PE-Cy5.5	710/50
	C	PE-Cy5	660/40
	D	PE Texas Red	610/20
	E	PE	575/25
	F		
	G		
	H		
628 nm 200 mW	A	APC-Cy7	780/60
	B	Alexa 700	730/45
	C	APC	660/20

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 CO_2 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.