

BOSTON COLLEGE CELL SORTING FACILITY

General Information

Location: BIOLOGY DEPARTMENT
HIGGINS HALL - ROOM 468
140 Commonwealth Avenue
Chestnut Hill, MA 02467

Cell Sorter Facility Manager: Patrick Autissier

Phone numbers: 617-552-1417 (Office)
617-552-1424 (Cell Sorter room)
617-552-1423 (Ante room)

Fax number: 617-552-2011

E-mail addresses: autissie@bc.edu (P. Autissier)

SORT REQUEST FORM

Investigator:

Principal Investigator:

Institution:

Phone: Fax:

E-mail:

First Sort Date: Time desired:

Duration of study; From:/...../..... To:/...../.....

Number of cell sorts needed:

Please note: If samples do not arrive at the scheduled time, we cannot guarantee that the specimens can be processed. Therefore, please contact the Facility ASAP if you experience any delays.

GENERAL RULES

A – All specimens must be transported in accordance with DOT/IATA regulations. In general, this will entail TRIPLE LAYER PACKAGING.

B – For ALL FIXED SPECIMENS, appropriate and reliable methods must be used to inactivate potentially biohazardous agents (e.g. freshly prepared formalin solution: 2% for 30 min*). These procedures must be performed VERY CAREFULLY; otherwise, samples that are considered to be inactivated, but in fact are not, can pose a serious health risk to laboratory staff.

C – ALL UNFIXED SPECIMENS, which will be submitted to our Staff, will be considered as potential biohazards. Therefore, they will be processed under BSL-2+ practices (e.g. complete protective clothing).

D – UNFIXED SPECIMENS, potentially infected with Hepatitis B or C or TB or Herpes Virus Simiae (B virus), will not be accepted for cell sorting.

E – PRINCIPAL INVESTIGATOR has to be accessible during the sorting procedure.

* For further details about Biosafety guidelines, you can refer to:

1. Schmid and Dean: Introduction to the biosafety guidelines for sorting of unfixed cells. *Cytometry* 28; 99-117, 1997.

2. http://www.isac-net.org/media/Biosafety_sorting_2007.pdf

B – Services required:

1 – Fluorochromes:

- | | | |
|---|--|---|
| <input type="checkbox"/> FITC/GFP/YFP (525nm) | <input type="checkbox"/> PE/RFP (575nm) | <input type="checkbox"/> ECD/PI (610nm) |
| <input type="checkbox"/> PE-Cy5 (665nm) | <input type="checkbox"/> PerCP-Cy5.5 (695nm) | <input type="checkbox"/> PE-Cy7 (780nm) |
| <input type="checkbox"/> APC (660nm) | <input type="checkbox"/> Alexa 700 (720nm) | <input type="checkbox"/> APC-Cy7 (780nm) |
| <input type="checkbox"/> Pacific Blue (455nm) | <input type="checkbox"/> QDot 605 (605nm) | <input type="checkbox"/> QDot 655 (655nm) |

Your panel/s:

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2 – Analyses:

- | | | |
|---|--|--|
| <input type="checkbox"/> Surface markers | <input type="checkbox"/> Ploidy | <input type="checkbox"/> Intracellular |
| <input type="checkbox"/> Gene expression | <input type="checkbox"/> Kinetic studies | |
| <input type="checkbox"/> Others (specify) | | |

3 – Sorting:

- | | |
|--------------------------------------|--------------------------------|
| <input type="checkbox"/> Live | <input type="checkbox"/> Fixed |
| <input type="checkbox"/> Sterile | |
| <input type="checkbox"/> Non-sterile | |

4 – Additional information:

- Number of cells in your sort sample (ideally 5-10 million/ml) resuspended in PBS only
Tubes (Falcon No 352054) should be a maximum of 2/3rd full.

- Cells should be filtered through the Miltenyi Biotec Pre Sep filter (yellow cones)
- Approximate % Sort Population(s) in sample:
- Approximate sorted cell number needed:
- Bring appropriate control samples to set up the cell sorter: negative control sample, cell or bead samples stained with each antibody used in your experiment individually
- Bring 12X75mm polypropylene tubes for collection of your sorted cells.
- Be sure to add approximately 0.5ml of appropriate medium (with 20%FBS) to each tube.
- Bring enough tubes to hold the number of cells you expect to collect.

If possible, please attach relevant previous flow cytometry data including forward vs. side scatter plot/s of the cell population to be sorted. List mode files on disk would be most useful. If appropriate, please attach previous publication(s).

Additional information

A – The cells to be sorted are of HUMAN ORIGIN

1. a - Is the human material:

- **A:** freshly isolated human cells (e.g. PBMC, cord blood, bone marrow)
- **B:** human cell lines or cultured cells
- **C:** human cells recovered from immunodeficient animals

• If **A:** freshly isolated human cells (e.g. PBMC, cord blood, bone marrow)

- Is the human donor infected with Hepatitis B or C viruses, or HIV?

- No
- Yes.....
-
-
-

- Does the donor have tuberculosis? (please note if tuberculosis is drug-resistant)

- Yes No
- drug-resistant Not drug-resistant

- Have the cells been treated to reduce the risk from infectious agents?

- No
- Yes.....
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-

• If **B:** human cell lines or cultured cells

- Please, describe the **origin of the cell line**, and indicate if it is infected with any transforming human viruses (e.g. EBV, HTLV-1, herpes saimirii):.....

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.....
.....
.....

- If the cells have been modified, describe the **vector** used to transduce them (if a retrovirus was used, show the vector map and describe the packaging cell line):.....

.....
.....

.....
.....

• If **C**: human cells recovered from immunodeficient animals

- Describe the prior recipient of human cells, and indicate if the cell population now contains both human and non-human cells:.....

.....
.....

Both

Only human cells

- Indicate if cells of any other species were also engrafted in the animal host:.....

.....
.....

- Were the engrafted human cells screened for human pathogens?

No

Yes.....

.....
.....

- If cells of other species were engrafted, indicate the potential for interaction between viral pathogens from that species and human cells:.....

.....
.....
.....

b - Is the material genetically modified?

No

Yes.....

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.....
.....
.....

B – The cells to be sorted are of NON-HUMAN PRIMATE ORIGIN

1. a - Describe the **primates species** from which the cells originated:.....
.....
.....
.....

b - Describe any known human pathogens that the primate species may harbor:.....
.....
.....
.....

c – Have the primate cells been exposed to or are infected with any agents infectious to humans (e.g. SIV, TB, Herpes Virus Simiae (B virus)) ?

- No
- Yes.....
.....
.....

- d** - Is the material being sorted:
- **A**: freshly isolated primary cells
 - **B**: cell lines or cultured cells
 - **C**: genetically modified cells

• If **B**:
- Please list any agents infectious to humans present in the culture:.....
.....
.....
.....
.....

• If **C**:
- describe the vectors and packaging cell lines used in genetically modifying the cell lines:.....
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.....
.....
.....
.....

C – The cells to be sorted are of MOUSE OR RAT ORIGIN

1. a - Are the **rodent cells** from an animal transgenic for an organism infectious to humans (e.g. hepatitis B or C, tuberculosis), or from an animal grafted with human cells?

No

Yes

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.....
.....
.....

b – Are the cells to be sorted Map (Mouse antibody production) or Rap (Rat antibody production) tested?

No

Yes

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.....
.....
.....

c - Are the cells to be sorted infected with any human virus or pathogen?

No
 Yes.....

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.....
.....

D – The cells to be sorted are of OTHERS SPECIES

1. Describe the **species of origin** of the cells (or cell lines) to be sorted. Please note any viruses capable of trans-species transmission with the potential to be considered a biohazardous agent:.....

.....
.....
.....
.....
.....

BIOHAZARD DECLARATION

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 chosen the appropriate status and understand that misclassification increases the risk to the staff of the Cell Sorting Facility and that action may be pursued for intentional misrepresentation.

I declare as such Biohazard status of my submitted specimen is:

- Unfixed, non-pathogenic
- Unfixed, pathogenic
- Fixed, non-pathogenic
- Fixed, pathogenic

In case non-fixed cells are sorted:

I declare that the specimens are to the best of my knowledge not infected with any of the following pathogenic agents:

Hepatitis B or C, TB, or Herpes Virus Simiae (B virus)

- specimens are negative for these pathogenic agents, because
 - humans or non-human primates have been tested to be negative
 -

specimens originate from an animal species that does not get infected by these pathogenic agents

Date:

Signature: