**Syracuse University Flow Cytometry Core Facility**

**Cell Sorting Standard Operating Procedure**

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**Purpose**

This standard operating procedure (SOP) is intended to provide a summary of the safety procedures in place for the Syracuse University Flow Cytometry core facility, as well as to outline the steps involved in transport, preparation, and running of samples brought by users on the Becton Dickinson (BD) FACSAria II Special Order Research Product (SORP) cell sorter in use by the facility, which is housed in a dedicated Baker BIOProtect Biosafety Cabinet. These procedures are intended to be specific to users of the facility. The practices of general laboratory safety, including those as outlined in the Syracuse University Chemical Hygiene Plan (CHP), should be adhered to at all times in the facility to ensure the safety of all facility users and personnel.

**Introduction**

The BDFACSAria II utilizes a flow-cell design which allows particles to move at relatively slow speeds while being interrogated by the relevant laser(s) (a process which occurs inside the quartz flow cell, rather than in the open as in traditional stream-in-air systems) before the stream is accelerated and drops break off to be sorted. The slow movement of particles in the flow cells allows the instrument to interrogate single cells individually and collect quantitative data about them based on fluorescent information interpreted by the software. The light scatter data can indicate information relevant to cell size and various aspects of cellular biology, including cell cycle stage, DNA content, gene expression, surface protein or receptor content, and more. While typical analytical flow cytometry works on the same basic principal, the cells in an analytical cytometer are sent directly to the instrument’s waste receptacle. A cell sorter does not send all of the analyzed cells to the waste; rather, the instrument makes a judgment, based on user-determined specifications, about how to separate cells into pre-define groups, and sends the cells from a mixed population into different collection receptacles based on phenotype. These sorted cells can then be collected and used for further analysis or even cultured.

**Biological Hazards**

Cell sorting is a high-throughput, high-speed process that has the potential to produce aerosolized droplets during the course of the experiment. Due to the potential for aerosols, potentially hazardous samples cannot be analyzed unless contained within the biosafety cabinet. Aerosol containment mechanisms both on the instrument and the biosafety cabinet minimize the escape of potentially hazardous aerosols during the course of an experiment; however, instrument failures such as clogged sorting nozzles or air in the fluidics lines can increase the risk of aerosolization by the instrument.

As mentioned, exposure to potentially hazardous materials can occur as a result of aerosols produced during use of the instrument. This risk is increased when working with samples which are unfixed or which contain infectious agents. This risk is highest when the operator accepts samples without gathering the most accurate information regarding the contents and procedures to which they have been exposed. Particular care should be exercised when considering samples of human or animal origin and those which have been exposed to recombination (viruses, nucleic acids, etc.). Samples which contain lentivirus, adenovirus, or other similarly engineered viral agents should be treated as a potential biohazard risk, and the appropriate Biosafety Level 2 (BSL-2) precautions should be exercised.

Additionally, cell sorting requires the use of a variety of fluorescent markers, many of which can pose a potential health hazard, whether they are toxic, mutagenic, teratogenic, or carcinogenic. Appropriate care must be exercised to minimize the risk of biological exposure through inhalation, mucous membrane absorption, etc. **No radiolabeled samples are accepted for sorting by the facility.**

Finally, generally laboratory precautions will be followed to minimize the risk of exposure from all samples. This includes safe use of equipment, consideration of appropriate biosafety level for each sample, and applicable treatment of samples based on biosafety level. Samples will never be vortexed in open containers; lids must always be secured before using the vortex. Similarly, although sample prep is expected to be completed prior to arrival at the facility, if there is some minor final preparation that needs to be addressed (pipetting to a new tube, filtration, etc), then biosafety level will be carefully considered. BSL-2 samples will only be uncapped within the biosafety cabinet, and never on the bench top.

**Biosafety Level**

|  |  |  |  |
| --- | --- | --- | --- |
| **Procedure** | **BSL-1** | **BSL-2** | **BSL-2+** |
| Cell type (description) | Cells from murine or other non-human / non-primate species that have **not** been exposed to any microbial agent **but have been**genetically modified using non-viral methods | Cells from human or non-human primates, cells that have been genetically modified using viral methods, or  cells exposed to microbial agents | Cells exposed to microbial agents with moderate increased personal hazard.  (e.g. West Nile Virus)  or cells that have been genetically modified using viral methods with known oncogenic inserts |
| Bloodborne Pathogen Training | Not required | Required | Required |
| Sort Signup | Required | Required | Required |
| Sample Transport | Leak-proof, double-walled container with secure lid | Leak-proof, double-walled container with secure lid and appropriate biohazard labeling on the outside | Leak-proof, double-walled container with secure lid and appropriate biohazard labeling on the outside |
| Room Restriction | Door closed while experiment in progress | Door closed while experiment in progress | Door closed while experiment in progress |
| PPE | Closed-toe shoes, long pants, gloves, and lab coat recommended | Closed-toe shoes, long pants, gloves, and lab coat **required** | Closed-toe shoes, long pants, double gloves, and lab coat **required** |
| Spills (PPED) | Gloves, lab coat, and eye protection **required** | Gloves, lab coat, and eye protection **required** | Gloves, lab coat, and eye protection **required** |
| Sample Waste | Gloves and other waste disposed of as biohazardous | Gloves and other waste disposed of as biohazardous | Gloves and other waste disposed of as biohazardous |

The biosafety level assigned to samples will be determined by EHS at Syracuse University; based on the assigned biosafety level, the appropriate SOP will be followed to ensure the safety of all personnel while handling the samples. As stated previously, radioactively labeled samples will not be accepted by the facility. In order to determine the acceptability of samples proposed for sorting, potential users will submit a detailed sort request questionnaire that will allow the operator to determine whether the proposed sample meets the requirements and restrictions applicable for sorting in the facility (appropriate biosafety level, no radiolabeling, no free virus particles in the sample, etc.).

**Facility Security**

The flow cytometery core facility will host operating hours of 9:00-5:30pm, Monday-Friday (subject to scheduling restrictions) and is located in 309A Link Hall. During operating hours, there will be a dedicated cell sorting operator present in the facility to operate the instrument and supervise the facility. At any time the operator is not present in the facility, the door to the laboratory will be shut and locked, restricting access to the facility materials and instruments. Key access to the facility shall be granted only to the operator and to the principal investigator (Dr Dacheng Ren, 357 Link Hall).

Once samples arrive at the facility for processing, the door to the facility will be closed and will lock from the outside. This will prevent the entry of unauthorized persons into the facility while experiments are in progress. Furthermore, while an experiment is in progress, the appropriate biohazard signs will be applied to the door to indicate the biosafety hazard level, the type of hazard present within the room, and entry/exit requirements for the facility. Within 309A, airflow moves in a unidirectional, inward fashion (negative pressure); this prevents the escape of biological agents in the event of an accidental spill. During the course of the experiment, hazardous samples will be handled only within the confines of the biosafety cabinet, which will be on with the blower running. This will further minimize the chance of escape of biological materials. Finally, since no user samples will be stored within the facility except for the duration of the experiment, there is no risk that hazardous biological material can be removed from the facility by unauthorized personnel in the absence of facility staff (i.e., through theft after hours).

**Sort Request and Approval**

Due to the fact that there is the potential for biological exposure from samples provided by facility users, the facility staff will thoroughly screen proposed sort requests prior to approval. The process for sort request and approval will be handled as follows:

1. Potential users will visit the flow core website and fill out the sort request form (flowcore.syr.edu/?page\_id=112)
2. The sort request form requires a variety of detailed information involving the sample and the experimental procedure(s) involving the sample which will help the operator determine whether or not it is appropriate to be handled by the facility. Information gathered by the sort request includes pertinent user contact information, sample type, presence of infectious agents, transformation status, genetic engineering performed, transfection information, and IBC approval, among other information more specific to the individual sorting procedure.
3. Upon completion of the sort request, the potential user will submit the request. The site will generate an automatic response, which will alert the user via the provided email that the request is being considered. Meanwhile, an alert will arrive in the operator’s inbox to indicate that a new sort request has been received. The operator will review the request and make the determination, based on the provided information, about whether or not the sort request will be approved or denied.
4. Once the approval determination has been made, the operator will notify the user via email about the decision. In the event of a denial of sort request, reasoning will be provided; however, acceptance of samples remains with the facility staff, who reserve the right to reject samples that are deemed inappropriate or likely to pose a danger as a result of handling by the facility.
5. If the sort request is approved, the user will receive an email notifying him/her of the decision. The email will contain all the pertinent information necessary for the user to safely prepare and transport samples for use in the flow core facility. The email will also provide instructions for reserving instrument time via the facility website.
6. Once approval has been granted, the user can schedule time on the facility website (flowcore.syr.edu/?page\_id=109). Before scheduling time, the user must fill out an additional form that requires reentry of pertinent contact information (must be valid for the user who will accompany samples to the facility) and information regarding the sample to be sorted, including whether any information has changed since submitting the sort request.
7. All submissions for instrument reservations must be reviewed by the operator. This ensures that no user can reserve instrument time without first having an approved sort request. Once the time is scheduled and approved, the user is ready to bring samples at the reserved time for analysis.

**Pre-Sort Preparation**

All sample preparation should be completed by the user at his/her own facility (i.e., at his/her own laboratory) prior to arrival at the flow core facility. Sample preparation refers to harvesting of cells, washing, filtration, and storage on ice, as appropriate for each sample type. Flow cytometry and, by extension, cell sorting, is most successful when the instrument is allowed to analyze single cells in suspension rather than clumps of cells, as often happens. Therefore, it is often necessary (depending on and varying by the type of cell and experiment) to wash cells, filter cell suspensions, and treat with agents such as trypsin and DNase to ensure that samples are suitable for cell sorting. Washing can also be necessary as part of the staining or cell preparation processes. It is preferable and encouraged that these procedures be completed in the users’ respective laboratories. However, it is acknowledged that, due to transportation time and factors such as settling, it may be desirable and even necessary to perform some limited sample preparation in the flow core facility.

For this purpose, the facility will have centrifuges, a vortex, and pipettes available to users. As previously mentioned, samples may not be vortexed in open containers; any sample which will be subjected to vortexing must be enclosed in a container with the lid securely attached to prevent the escape of sample into the facility. Furthermore, the biosafety level of the sample will be carefully considered before any manipulation of sample is allowed within the facility. Any sample which is BSL-2 or above will not be permitted to be opened outside of the biosafety cabinet; this will prevent aerosolization of the sample inside the laboratory and minimize the risk of exposure to biologically hazardous materials.

**Sample Transportation**

All samples must be transported to the facility in sturdy, leak-proof double-walled containers (at least one layers must be leak-proof) capable of preventing escape of the sample in the event of breakage or spill of the sample tube(s). The container must have a lid that fastens securely during transport to further prevent escape of sample in the event of an internal spill. Prior to leaving his/her own facility, the user should wipe down the outer surfaces of the transport container with disinfectant (for example, 10% bleach solution) to sterilize it. The outer surface is now safe to touch, regardless of biosafety level, and the user should not use gloves to carry the container to the facility. Finally, prior to leaving his/her own facility, the user should affix the appropriate biohazard warning label to the outside of the container. The label should clearly indicate the biosafety level appropriate for the samples. The container should remain securely closed until the user reaches the facility.

**Facility Preparation Procedures**

Prior to a user’s arrival at the facility, there are several tasks that need to be completed to ensure that the instrument is functioning properly, adequately prepared and ready for the sort, and that the area is clean and ready for sorting. The following details the steps required to prepare the facility for each individual sorting experiment:

1. At the beginning of each day, a volume of approximately 500 mL of pure bleach (0.5% hypochlorite) will be poured into the waste container. This volume ensures that, when the waste container is full, bleach comprises approximately 10% of the container’s total 5 L volume.
2. The operator will ensure that the sheath tank is full of sheath fluid.
3. The computer will be started, the cell sorter will be turned on, and the software will be launched.
4. Turn on the biosafety cabinet and the blower, and allow both to run so that their function is verified by the end of setup.
5. After the instrument (lasers) has been allowed to warm up, the quality control function of the software (CS&T: Cytometer Setup and Tracking) will be launched. Instrument function will be assessed using cytometer-appropriate beads, and the software will be used to ensure that laser delay and area scatter are within the recommended limits set by the software.
6. Use the Accudrop beads to test the stability of the side streams and the drop delay/drop breakoff. Adjust as needed either manually or using the software’s automated drop delay function.
7. Test side streams using the collection device which will be employed for the upcoming sort. Side streams should be adjusted so that the streams are deposited neatly within the collections tubes and do not splatter by hitting the tops or sides of the collection tubes. Splattering of side streams leads not only to contamination between samples but also to reduce the incidence of additional aerosolization that can arise from misplaced side streams.

**Sample Sorting Procedures**

Only the operator will be permitted to use the instrument for cell sorting; this restriction will minimize the risk of instrument and sample mishandling, which has the potential for allowing exposure to biologically hazardous materials. Users will transport the samples and, if necessary, perform minimal sample preparation in the facility as discussed previously. Prepared samples will be handled by the operator for analysis with the sorter. The operator will adhere to a set of protocols designed to contain the sample within the confines of the biosafety cabinet and minimize exposure to any biological samples. These procedures are outlined in the following.

1. Samples will not be uncapped on the bench top. All samples will be placed inside the biosafety cabinet (with the glass shield all the way down and secured) prior to opening. Once a sample is opened, it will be placed onto the stage. The sample access door will then be closed, and the sample will be loaded into the instrument (via the software).
2. There are two other points from which sample can escape from the instrument during the sorting process: the flow cell access door, and the sort block chamber door. When these are shut, there is little chance of sample escape from within the confines of the instrument. Consequently, these access doors will remain closed while samples are being processed. The sample access door will only be opened to insert and remove samples, while the sort block chamber door will only be opened at prior to and following the sort in order to insert and remove collection tubes.
3. Prior to beginning the sort, the operator will run a sample of buffer (to establish the instrument threshold in relation to the sample particle size) and a small sample of mixed population in order to visualize the sample in the software and set the appropriate gates. The user will be present during this step in order to assist in gate-setting determination and review of the sort logic. Once this step is completed, the user can either stay to observe the sort or leave. If the user decides to leave during the sort, a valid phone number must be provided to reach him/her in the event that the user must be contacted during the sort. When pre-sorting is complete, samples can be loaded in the instrument.
4. Once samples are loaded in the instrument and all access doors are closed, the sort will be started using the software. For the duration of the sort using a particular sample, the sample will remain enclosed in the instrument. Upon completion of the sort (determined either by pre-set, user-defined threshold values in the software or by manually stopping the run using the stop feature in the software or the emergency shutoff button located on the instrument) the sample will be unloaded from the instrument and the stage will return to the bottom of the sample access area so that the door can be open and the sample can be removed.
5. When a sample is unloaded from the instrument, several precautions will be undertaken:
   1. One minute will be allowed to elapse for aerosol evacuation
   2. The sample tube will be removed from the stage and recapped.
   3. The outside of the sample tube will be wiped with 10% bleach to disinfect.
   4. The tube can then be removed from the biosafety cabinet and placed directly in the transport container with the lid secured.
6. Samples that are handled by the cell sorter are separated into up to four distinct tubes. Prior to being removed from the biosafety cabinet, these sorted samples are analyzed on the instrument (post-sort analysis) to determine sort purity and efficiency. Post-sort samples will be handled according to the following protocol:
   1. Upon completion of the sort and after the mixed population sample has been removed from the instrument (see step 4), the sort block chamber door will be opened and the sort collection device will be removed. The sort block chamber door will then be closed.
   2. The tubes will immediately be capped to contain the samples and prevent contamination. Each post-sort sample will, if desired by the user, be post-sort analyzed by the operator.
   3. The post-sort tube will be uncapped and loaded onto the stage and the sample access door will be closed. The sample will be loaded into the instrument using the software and post-sort analysis will be completed (again, using the software).
   4. When the post-sort analysis is finished, the sample will be unloaded, and the sample access door will be opened.
   5. Similarly to the procedure for the mixed population sample, the post-sort sample tube will be immediately recapped. The outside of the tube will be wiped with 10% bleach to disinfect, and then the tube will be removed from the biosafety cabinet and placed immediately inside the transport container with the lid secured.
   6. Steps c-f will be completed for each of the post-sort tubes.

**Post-Sort Procedures**

Following completion of sorting and post-sort analysis, the user may participate in some data review with the operator. After data has been reviewed, the user will transport the sample from the flow core facility back to his/her own facility. Following the user’s departure, the flow core staff will complete decontamination procedures as follow:

1. A tube containing 5 mL of 10% bleach will run through the instrument for 10 minutes. This will be followed by a tube containing 5 mL of sterile, distilled water for 10 minutes. These two runs will serve to decontaminate the sample lines inside the instrument.
2. The sort chamber, sample chamber, sort collection device, and immediate surrounding surfaces will be wiped with a 10% bleach solution.

**Sample Waste**

The facility will handle both liquid and solid waste related to sorting biological samples. Liquid waste will largely be limited to the contents of the instrument’s waste container, as the user will be taking samples (both pre- and post-sort) back to his/her facility following the sort. In the unlikely event that there is liquid waste remaining in the facility that is not removed by the user, it can be disposed of by diluting with fresh bleach (0.5% to a total volume of 10% bleach) and leaving the sample in contact with the bleach for at least 30 minutes. Since bleach is added to the waste tank at the beginning of the day, sample that ends up in the waste is immediately exposed to bleach. At the end of the day, the waste tank can be emptied into the sink; following disposal, water should be flushed into the sink for at least one minute (**note:** when disposing of liquid waste and the filter cap is removed, do not allow the filter cap to get wet. This can damage the cap and prevent it from containing liquid waste properly. Set the filter cap label-side up until it is necessary to return the cap to the waste container).

Solid waste generated in the facility will be generally limited to tubes and pipette tips used to prepare samples, as well as for other reagents related to the preparation and use of the instrument (CS&T, Accudrop, etc.). Other solid waste might include paper towels and gloves that have come into contact with potentially biohazardous materials. Solid waste will be collected in red biohazard bags (clearly displaying the biohazard symbol) which themselves will be situated in leak-proof containers. When a biohazard bag is nearly full, it will be sealed, and autoclave indicator tape will be applied. The bag will be autoclaved (at least 121°C for 90 minutes), and the autoclaved waste will be scheduled for pickup by EHO.

**Spills**

In the event of a spill on the benchtop or floor of the facility, the university community is protected from potentially hazardous materials by the negative pressure in the laboratory. Depending on the biosafety level assigned to the sample, the appropriate PPE should be donned before attempting to clean the spill (see PPE in the **Biosafety Level** section). Once the operator is wearing the proper PPE for the sample type, cleanup can proceed. The operator should cover the spill with paper towels and then apply 10% bleach solution around the edges of the spill and directly onto the paper towels covering the spill. The bleach should be allowed to remain in contact with the spill for at least 20 minutes. After 20 minutes have elapsed, the paper towels can be picked up and disposed of in the biohazardous waste container. The affected area should then be cleaned again with 10% bleach, with the paper towels again being discarded in the biohazardous waste container.

In the event of a spill in the biosafety cabinet, the facility is protected by the airflow in the cabinet. The procedure to clean a spill in the biosafety cabinet is the same as the procedure to clean a spill that occurs outside of the biosafety cabinet. The operator should don the appropriate PPE for the sample type, cover the affected area with paper towels, and apply 10% bleach solution to the edge of the towels and onto the spill area covered by the towels. As above, 20 minutes should be allowed to elapse before the paper towels are cleaned up. The spill should then be cleaned again with 10% bleach. All paper towels associated with cleaning a spill of biohazardous material should be disposed of in the biohazardous waste container. Any gloves used to clean a spill should also be disposed of as biohazardous.

**Exposure**

An "exposure incident" is specific contact (eye, mouth, other mucous membrane, respiratory tract via inhalation, non-intact skin, or parenteral) with potentially infectious materials that results from the performance of a worker's duties. An employee who sustains a known or potential exposure incident must remove gloves and treat the affected area immediately Eyes exposed to hazardous materials should be flushed with water for 15 minutes. Skin exposed to hazardous materials should be thoroughly washed with soap and water. Mucous membranes exposed to hazardous materials should be flushed with water for 15 minutes. Workers must report any unanticipated exposure, potential exposure, accident, or injury received on the job to his/her supervisor. The supervisor must complete an Injury Report form documenting the route of exposure and the circumstances under which the incident occurred and provide a copy to Risk Management and the Biosafety Officer. A copy of the Accident/Illness Report Form can be found online at the following web-page. <http://riskmanagement.syr.edu/RiskManagement/display.cfm?content_ID=%23%28%28%25%2D%0A>

**Unexpected Stream Shutoff During Sorting**

The stream runs continuously while the instrument is turned on and in use unless the operator turns if off in the software. However, the stream can stop unexpectedly due to clogs in the sample line tubing or the nozzle. This not only disrupts (completely inhibits) sorting, but also increases the chance of unintended aerosolization. Clogs and sort disruptions need to be recognized and resolved as quickly as possible to return sorter function to normal. In the event of a clog, the sorter is designed to stop automatically, block the sort tubes, and evacuate the sample chamber; the instrument will not restart until the clog is removed.

In order to resolve a clog, the following procedure should be used:

1. Personal protective equipment appropriate to the sample type should be employed, and face/eye protection should also be utilized.
2. The blower in the biosafety cabinet should be on before addressing the clog.
3. The sample should be removed from the sample chamber and recapped. The tube should be disinfected with 10% bleach and removed from the biosafety cabinet; upon removal from the biosafety cabinet, the tube should be immediately returned to the transport container and the lid should be secured.
4. The sort collection chamber door should be opened, and the sort collection device should be removed. The tubes should be immediately capped, and the collection device should be wiped down with 10% bleach. Tubes can remain to the side of the biosafety cabinet.
5. The inside of the sort chamber and the sample chamber, as well as the surrounding surfaces should be disinfected with 10% bleach (contact time of at least 3 minutes).
6. The operator will turn the stream on to see if the clog has been resolved. If the stream is still not operational, the operator should clean the nozzle. The nozzle is the most likely source of the clog and should be addressed first.
   1. Turn off the stream again and open the flow cell access door.
   2. Turn the nozzle-locking lever and remove the nozzle.
   3. Soak the nozzle in a test tube of sterile, distilled water.
   4. Reinsert the nozzle in the flow cell and return the nozzle-locking lever to the locked position.
   5. Turn on the stream and make sure if flows properly.
   6. If the stream does not flow properly, remove the nozzle and sonicate for 5 minutes.
   7. Reinsert the nozzle in the flow cell and return the nozzle-locking lever to the locked position.
   8. Turn on the stream and make sure if flows properly.
7. If the clog is not resolved by cleaning the nozzle, the clog is in the sample line and the line will have to be replaced.

**Quality Control and Instrument Maintenance**

Regular quality control and maintenance of the instrument ensures that it functions properly; a properly functioning instrument provides the absolute minimum chance of malfunction and subsequent exposure to biohazardous materials. Quality control and maintenance in the facility will include the following:

1. The operator will perform CS&T (see **Facility Preparation Procedures**) to maintain an accurate log of cytometer function and to ensure that, prior to each sort, the cytometer is within the recommended laser delay and other parameter ranges for the current configuration.
2. The waste tank will be emptied at the end of each day. Since bleach is added to the waste container each morning such that the final bleach concentration in the waste container will be 10% by volume (see **Facility Preparation Procedures**) and the waste container sits throughout the day while the instrument is in use, any sample that goes to the waste container is inactivated by prolonged contact with the bleach. The contents of the waste container can thus be disposed of in the sink. After disposing of the contents in the sink, the sink should be flushed with water for at least 1 minute.
3. Instrument flow cell will be cleaned daily to diminish contamination.
4. Instrument will be subjected to a weekly ethanol cleaning to clean the sample pathway.
5. Prior to sorting, the sorter and the biosafety cabinet will be checked for cracks, leaks, and signs of stress. Any indication that either is not functioning properly will be cause for the sort to be postponed.

**Sort Request Form**

Instructions This form is for new users AND for those users who have previously used the facility but are bringing a new type of cell to be sorted. Please note that this request form is for the BDFACSAriaII cell sorter, NOT the BDAccuri C6 flow cytometer. Please fill in all required fields with accurate information and submit for review by the flow core staff. Your sort request will be reviewed as quickly as possible and you will be notified whether your samples will be sorted. Pending sort request approval, you will be given instructions on how to reserve time on the machine. Please be sure to provide accurate information on this form and to review the facility policies. Thank you for your interest in our facility. We look forward to working with you!

1. Principal Investigator \*First and Last Name
2. Investigator Name (First and Last) \* (Who will bring the samples to the flow core.)
3. Institution or Company \*
4. Phone Number \*
5. Email \*
6. Has this protocol received IBC approval? \*

YesNo

1. If yes, please attach a copy of your approval letter using the link at the bottom of the form. 
2. Please indicate the type of cell to be sorted: \*



1. Please indicate the number of samples: \*



1. Please indicate the fluorochrome(s): \*

FITCPerCP-Cy5-5PE-BlueAPC-Cy7APCAlexaFluor 700AmCyanPacific BluePE-Cy5.5PE-Texas RedPE-Cy7PE-Cy5PEGFPYFPOther

1. If other, please specify:

(You will be notified as to whether the fluorochrome(s) is/are acceptable.)

1. How many cells to record for analysis (events)? \*



1. Please estimate the concentration of the sample(s) you will bring: \*



1. Number of populations to sort per sample: \*



1. Concentration of population to be sorted \* (Or relative abundance)
2. Pre-sort sample temperature \*

Room Temperature4C37C

1. Post-sort sample temperature \*

Room Temperature4C37C

1. The sample(s) will be sorted into: \*

TubesPlatesSlides

19. If you indicated that your sample(s) will be sorted into plates, please indicate the number of wells in the plate. \* (i.e., 96 wells) 

20. Please indicate the approximate size of the cells in your sample: \*



21. Which nozzle would you like to use? \*

70um85um100umunknown

22. If unsure about which nozzle to choose, please indicate: \*

operator can be contacted at gaaltimu@syr.eduI would like the operator to choose the appropriate nozzle sizeI will contact the operator to discuss choice of nozzle

23. Are the cells in the sample fixed? \*

YesNo

24. If yes, please indicate the fixation method:



25. Is this cell line adherent? \*

YesNounknown

26. Has the sample been treated with trypsin? \*

YesNo

27. If yes, has trypsin been inactivated?



28. Has the sample been treated with DNase? \*

YesNo

29. Please list all infectious agents present in the sample: \*



30.Were these cells transformed using any virus (EBV, HTLV-1, etc)? \*

YesNo

31. If yes, please specify:



32. Were the cells genetically engineered? \*

YesNo

33. If yes, and a virus was used, please descibe the method in detail.

Please attach a vector map using the link at the bottom of the form.

34. Have these cells been trasfected with a virus, nucleic acid, viral vector, or any other pathogen? \*

YesNo

35. If yes, please specify:



36. Please attach a copy of your IBC approval letter, if applicable.

37. Please attach a copy of your vector map, if applicable.

**References**

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of Unfixed Cells. Cytometry Part A 71A:414-437. 2007

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