CytoFLEX SRT Experimental setup form - Initial setup

Updated: 06-20-2023

Auburn University Flow Cytometry and High-Speed Cell Sorting Laboratory
Location: 250A Greene Hall, Auburn, AL 36849
Contact: rzw0041@auburn.edu (Rie Watanabe, Operator & Manager) birdric@auburn.edu (Dr. Bird, Director)
Lab phone#: 334-844-2711
Operation hours: 800am-445pm (M-F)

****Please read through this document and contact us with any questions before you submit sorting request****

Scheduling:

- Sorting can be started between the hours of 900am – 330pm. If sorting outside of the regular hours is required, the request must be discussed and approved before the submission.
- A minimum of 30 minutes before or between sorts is required to clean the machine
- A minimum of 1 hour is required before or between sorts is required if aseptic cleaning is requested
- For scheduling, email rzw0041@auburn.edu. Sorting must be scheduled a minimum of 1 working day before the requested appointment time to ensure the scheduling

Sample requirement and preparation tips:

- Minimum volume is 0.5ml even if this volume does not give the ideal cell concentration
- Desired concentration of cells is 1~10 x10^6 cells/mL (after filtration)
- Samples are supposed to be single cell suspension only (NO CLUMPS). Should be filtered through cell strainer to make good single cell suspension (increases sort efficiency!) and prevent clogging
- Recommended sorting buffers are 1xPBS (free of Ca^{2+}/Mg^{2+}), Hank's. Buffers can be supplemented with BSA (0.1-1%) or dialyzed FBS (1-5%). Addition of EDTA (up to 5mM) or DNaseI (25-50ugml, along with $MgCl_2$) may help preventing cells clumping via DNA released from dead cells
- Always prepare the unstained control. Cell numbers don't have to be as high as your sorting samples but it is essential to set the gates
- If your samples are stained with more than 1 fluorochrome, prepare samples stained with each fluorochrome individually. This is for compensation purposes

Sample and Collection containers:

- Prepare samples in polyprolylene 5ml (12x75mm) round bottom tubes suit our instrument best
- Sorted cells can be collected in 15ml conical tube, 5ml (12x75mm) tube, or 96-well plate
- Prepare your collection containers pre-loaded with receiving solutions (media etc)

Expectations for cell sorting:

- Sorting time depends on your objective and sample quality. If you need more information, see Appendix or contact us
- Expect the final yield is about 75% of the starting number of desired cells. For example, 1x10^7 cells with 30% GFP(+) would yield 2.25 x10^6 GFP(+) cells (10^7*0.3*0.75). Rare event sort (1% or less) can produce a lower yield, as low as 50%. It will be diluted as well since sorted cells takes up sheath

Submitter information

Date:	**Start/Finish:**	
Researcher:	**Contact information**	**Email:**
Lab/PI:		**Phone:**
Department/College/Institution:		
Billing contact:		
If outside of the Auburn University, Street address and City:		

Sample information (check all applied)

Cell type: ☐Cell line ☐Primary cells ☐ Other_______________________

Species: ☐Human ☐Non-human primate ☐Mouse ☐Rat ☐Other ________________________

Tissue Origin: ☐Bone Marrow ☐Peripheral Blood ☐Neural ☐Other________________________

Adhesiveness: ☐Non-adherent ☐Adherent

Mode of Disaggregation: ☐Trituration ☐Trypsin ☐Collagenase ☐Other________________________

Size of cell strainers used: ☐70um ☐50 um ☐30um ☐Other________________________

Estimated cell size: ☐<10um ☐10-20um ☐20-70um ☐70um<

Number of starting cells: ________________________

Volume of cell suspension at start (ml): ________________________

Solutions cells suspended: ☐PBS ☐HEPES ☐Hanks ☐BSA____% ☐Other________________________

Do samples potentially contain infectious agents? ☐Yes ☐No
→If yes, what infectious agents? _______________________

Do samples contain recombinant or synthetic nucleic acids? ☐Yes ☐No
→If yes, how was your nucleic acid introduced to your samples? ☐Transfection ☐Viral vector
→If yes, do those genes encode any recombinant proteins with potential oncogenic properties? ☐Yes ☐No

What is the appropriate Biosafety level of the samples? (See appendix A) ☐BL1 ☐BL2 ☐BL2-enhanced

BUA accession number by IBC, Auburn University or your institution ________________________

---Flow lab use only---

BUA confirmed	
Sorting BUA level	
AU flow lab sorting experiment #	
Comment	

Sorting channel information (select all applied)

Light scatter: ☐FSC ☐SSC

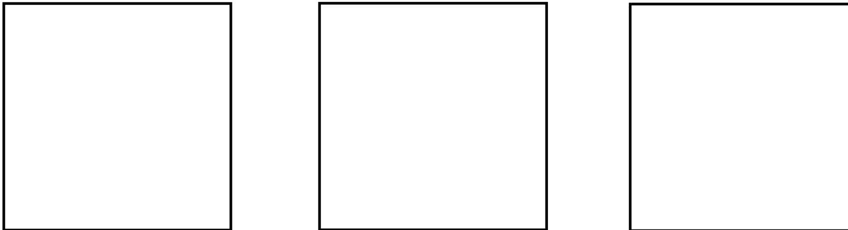
Blue laser (488nm): ☐B525-FITC ☐B690-PC5.5

Yellow laser (561nm): ☐Y585-PE ☐Y610-mCherry ☐Y675-PC7 ☐Y710-PC5.5 ☐Y780

Red laser (638nm): ☐R660-APC ☐R712-PCA700 ☐R780

Violet laser (405nm): ☐V450-PB ☐V525-KrO ☐V610 ☐V660 ☐V780

Sorting Schema (please show fluorescent patterns, gating parameters, and hierarchy of target cells and subsets)



Sample information (for reservoir position and sorting mode, see Appendix B and C)

Tube ID	Sample	Position*, reservoir	Sorting mode**	Initial vol	Target population
Example	Ex	☐**Plate** ☒**L2:** ☒15ml ☐5ml ☐**L1:** ☐5ml ☐**R1:** ☐5ml ☒**R2:** ☐15ml ☒5ml	☐Single cell ☐P ☒P1-2 ☐Enr ☐P ☐P1-2 ☐Enr ☐P ☐P1-2 ☐Enr ☒P ☐P1-2 ☐Enr	______uL ___2__mL ______mL ______mL ___1__mL	________________ Middle of GFP(+) ________________ ________________ Top 5% of GFP(+)
1		☐**Plate** ☐**L2:** ☐15ml ☐5ml ☐**L1:** ☐5ml ☐**R1:** ☐5ml ☐**R2:** ☐15ml ☐5ml	☐Single cell ☐P ☐P1-2 ☐Enr ☐P ☐P1-2 ☐Enr ☐P ☐P1-2 ☐Enr ☐P ☐P1-2 ☐Enr	______uL ______mL ______mL ______mL ______mL	________________ ________________ ________________ ________________ ________________
2		☐**Plate** ☐**L2:** ☐15ml ☐5ml ☐**L1:** ☐5ml ☐**R1:** ☐5ml ☐**R2:** ☐15ml ☐5ml	☐Single cell ☐P ☐P1-2 ☐Enr ☐P ☐P1-2 ☐Enr ☐P ☐P1-2 ☐Enr ☐P ☐P1-2 ☐Enr	______uL ______mL ______mL ______mL ______mL	________________ ________________ ________________ ________________ ________________

If needed, add rows

Checklist on your sorting day

- Cells suspended in sorting solution, prepared in round bottom 12x75mm tube, 5ml (Ex. Falcon 352058)
- Receiving tubes pre-filled with necessary amount of media or transporting solution (15ml or 5ml tube)
- Transporting container (Need to meet the requirement of your BUA)

Appendix

A. Examples of Biosafety level of samples

	BL1 exempt	BL1	BL2
Origin of cells	murine or other non-human, non-primate species	Most, excluding human, non-human primates	human, non-human primates, others
Infectious agent	NO	No	Yes (BL2)
Recombinant nucleic acid	NO	Yes (tfxn, TG)	Yes
Viral vector	NO	No	Yes
BUA number	not required	**required**	**required**
PPE	Recommended	recommended	**required**

B. Differences in reservoir position (5-71)

Position	Reservoir accommodated	Benefit, suitable for
Plate	Multi-well plates	Single-cell cloning
L2 (outer left)	15ml, 5ml	Provides maximum purity. Recommended for precious/rare events to be sorted in.
L1 (inner left)	5ml	Macro-particles (>=15um) are recommended to go here
R1 (inner right)	5ml	Macro-particles (>=15um) are recommended to go here
R2 (outer right)	15ml, 5ml	Provides maximum purity. Recommended for precious/rare events to be sorted in.

C. Differences in sorting mode (2-47)

Mode	Definition and major applications	
Single cell	Definition	Having only one event per drop is the most important aspect of the sort.
	Application	single cell cloning (plate/slide sorting)
P: Purity	Definition	The purity of the sort is the most important. Can gate the % of cells wanted.
	Application	Harvesting top XX% positive cells
P1-2: Purity 1-2	Definition	Same with “Purity” but more inclusive than Purity mode.
	Application	Get sorting done a little faster (sacrificing purity slightly).
Enr:Enrich	Definition	Recovery is the most important aspect of the sort.
	Application	Collecting all positive events even it contains negative events in or near the drop.