

## **Duke-NUS Cell Sorting Core Facility**

Cell Sorting Form

Number of populations to sort:

Prepared by:	DUKE-NUS Research Operations			
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Tel:	65-66016287			
Effective from:	15 May 2019			

cen sorting rotti		Tel:	65-6601628/			
		Effective from:	15 May 2018			
Customer Information:						
PI Name:	PI Email:					
Department & Institute:						
Billing Address:						
Requestor Name:		Requestor's				
Requestor Email:		Phone:	Phone:			
Fund/Grant Code (NCC):		WBS Account (Duke-NUS):				
Date/Time service required: (dd/mm/yyyy)	g (/)	□ Af	ternoon (/)			
Operating hours: week days 10am-1pm (morning) and 2	pm-6pm (afternoon)					
Sample Declaration (Requestor to provide	information):					
Are any of the samples:						
1. Of human origin?	Please indicate species, source and clinical history of specimen.					
☐ Yes ☐ No	Species:	Species:				
	Source:					
2. Of non-human primate origin?						
☐ Yes ☐ No	Clinical History:					
a. If yes, are the animals negative f Herpesvirus B or SIV (Simil Immunodeficiency Virus) infection?						
☐ Yes ☐ No						
Obtained from human subject undergoing febrile illness of unknown origin?	vn Immunodeficien	cy Virus), HCV us) or any known	5. Characterized by active viral replication (eg. <i>In vitro</i> infected cell lines)?			
□ Yes □ No	□ Yes □	No	□ Yes □ No			
6. Transduced with plasmid or viral vecto	ors? *If Q5 or Q6 is yes, pleas	e specify vector and/c	or infecting virus.			
_						
Sort Details (Requestor to provide informa	tion):					
	ozzle size: 70 / 100 micron	Cell Type:	Fixed / Unfixed samples			
355nm (UV): □ BUV737		JV395				
405nm (Violet): □ BV78	86 □ BV711 □ Qdot	655/BV650 □ B\	/605 □ BV480 □ BV421			
Colors: 488nm (Blue): 🗆 FITC/#	488nm (Blue):   FITC/Alexa488/GFP   PerCP-Cy5.5/7AAD					
561nm (Yellow-green):	561nm (Yellow-green):   PE RFP/mCherry/PE-CF594 PE-Cy5 PE-Cy7					
635nm (Red):   APC/A	lexa647 🗆 APC-Cy7					
Total number of cells: (pre-sort):	% of	population of inter-	est: Concentration (Cells/ml):			



Service(s) Provided (to be filled by FACS operator):								
Cell sorting FACS Aria	II at discounted rate of	\$100 per hour (exc	luding 7% GST) (Usual rate:	\$130 per hour)				
Date	Start Time	End Time	Total No. of Hours	Unit Price (\$)	Total Cost (\$)			
				100.00				
				Grand Total				
				(excl GST)				
Confirmation of Orde	er:							
the cell-sorting facilit		d above is correct a	nd that I have read and un	derstood the terms and	conditions for using			
FACS Operator / Date			Signature of PI / Date					
Acknowledgement of	f Services:							
Service Completion D	Pate (dd/mm/yyyyy):							
I hereby acknowledge	ed that the service has b	een completed.						
FACS Ope	erator / Date	Signature of Requestor / Date						
For Internal Refe	rence Only							
Billing Information:								
Invoice Number/Date	2:		Payment Date:					
Verified By:								



## Terms and conditions:

- 1. Contact *Charlene Foong Shu Fen or Daniel Anand Silva at <u>facs@duke-nus.edu.sg</u> to plan a sort every time you want to do a new type of experiment to discuss the requirements and set-up of the machine.* 
  - 2. Operation of the machine is strictly performed only by the core facility operators.
  - 3. Charges for the use of this facility is \$100/hour excluding GST.
  - 4. Sorts need to be scheduled at least 5 days before. A cancellation fee of \$100 applies for cancellations made less than **24** hrs prior to sorting. Users have to send a cancellation email to facs@duke-nus.edu.sg at least 24 hours prior to the time of the booking (preferably earlier so we can reschedule).
  - 5. Please notify us as soon as possible if there are changes to the sort details provided in the form.
  - 6. Requestors are responsible for providing:
    - The necessary controls to ensure the proper gating and identification of the desired cell populations.
    - Collection tubes containing the appropriate media for the sorted cells.

## Sample preparation guidelines:

- 7. Samples have to be filtered through a 40 µm cell strainer to prevent clogging of the nozzle.
- 8. For standard sorts, a concentration of 10 million cells/ml is recommended (3-20 million cells/ml is the tolerable range for sorting).
- 9. Final cell yield is dependent upon many factors: starting percent of target population, total number of live and dead cells in the sample, cells stickiness, etc.
- 10. For improved cell yield, collection tubes can be pre-blocked overnight with media containing 10% FCS. The use of polypropylene instead of polystyrene tubes for collection can help to reduce cell stickiness.
- 11. Use of antibiotics in the collection media is recommended to prevent contamination but requestors should determine whether it is appropriate based on its effect on the sorted cell population.
- 12. Important information to note:
  - Size of your cells: to select the proper nozzle size
  - Cell Adhesiveness: to select resuspension buffer to minimize clogging
  - Total cell number: to achieve optimal sorting concentration (minimal clumping, maximum speed)
  - Approximate percentage of your target population: to determine sorting time and maximum possible yield

## **Biological hazards:**

- 13. All potentially biohazardous sorts and protocols will have to be pre-approved by PI in charge.
- 14. Due to biosafety reasons we will not sort:
  - Samples containing lentiviral vectors
  - Radioactive or radioactive labelled samples
  - Human samples
    - i. Undergoing a febrile illness of unknown origin
    - ii.Infected with HIV, HCV or any BSL3 pathogen
    - iii. Characterized by active viral replication (eg. In vitro infected cells)
  - Mammalian / murine samples
    - i. Undergoing infections of unknown origin
    - ii. If infected by human pathogens, the same rules described above will apply
    - iii.Non-human primate cells infected with Herpesvirus B or Simian Immunodeficiency Virus