BD Influx Standard Operation Protocol -Basic Operation

Optimizing the drop phase

- 1. Verify the 2-tube holder with dummy 15ml tube is in place on the sort stage
- 2. Select '2 tube holder 2 way sort' as the sort device in the Sort Layout pane.
- 3. Click '**Sort Ready'** in the *Sort Layout* pane to place the dummy in place.



Close the deflection plates and press 'Plate' to charge them
 Caution: Risk of electric shock if touch the deflection plates when they are charged.



5. Confirm the sort chamber door is closed. Click '**Test Streams**' in the *Sort Setting* pane to initiate test sort.

ort Settings			
Drop Formation			
Piezo Amplitude:	434 🔆	L.	Drop Drive
Drop Frequency:	89.20 음	кнг [V ON
Stream			
tream Focus:	11.20 🔶	1 %	
Maximum Drop Chi	arge (Volts)		
0 30 60	90 12	0 150	
-		0-	130
Tect Streams	lash Charge	Cheve F	inter l

6. Click 'Flash Charge' and adjust the Piezo Amplitude so that the side streams are maximally deflected with no fanning.



7. Click '**Short Flash**' and fine tune the **Piezo Amplitude** so that the side streams are maximally deflected with no fanning.

Drop Formation		
Piezo Amplitude	: 3.94	Drop Drive
Drop Frequency	39.20 ᠿ КН	z ON
Stream		
Stream Focus:	11.20	%
Maximum Drop C	Charge (Volts)	
0 30 60	0 90 120	150
-		130

8. Click 'Test Streams' again to turn off.

Accudrop Test

1. Click File > Restore > Workspace. The following window will appear.



Storage: Choose Default

Choose the Accudrop template according to the nozzle size.

2. Uncheck "Restore Laser Delay" and "Restore Fludics Setup"



3. Turn the ND filter knob to AccuDrop filter.



4. Load a tube of AccuDrop beads, press 'Sample' button beside the sample loading port and click 'Acquire' on the Acquisition Dashboard.



5. Adjust the **Sample Offset** to reach an event rate of <u>between 1000 to 3000 events per second</u>. (For 100um, event rate should be around 2000 events per second)



- 6. Check if 'Plate' button is on. Turn it on if needed.
- 7. Click 'Start' in the Sort Layout pane.



- 8. Choose **Sort Mode 2.0 drop Enrich** in the *Sort Settings* pane.
- 9. Adjust the '**Drop Delay**' until the left stream is the brightest while nearly no fluorescent signals can be detected in the mainstream position.



Wrong Drop Delay

Optimal Drop Delay

- 10. Choose **Sort Mode 1.0 drop Enrich** in the *Sort Settings* pane and repeat Step 7 to fine tune.
- 11. Click '**Stop**' in the *Sort Layout* pane and '**Stop**' on the *Acquisition Dashboard*.
- 12. Press 'Plate' to turn off the deflection plates.
- 13. Unload the tube and press 'Backflush'. Allow the flush for at least 5 seconds.



14. Turn the Accdrop filter knob back to ND filter position.



Attention: If you would like to further culture your collected cells, it is recommended to clean the sample line with FACSClean for 8 minutes at 27.5psi followed by MilliQ Water of same conditions after the Accudrop Test. BSC hood is recommended to be turned on.

Experimental Set up

1. Click *File > Restore > Workspace*. The following window will appear.

😂 Restore Workspace	
Restore Workspace	
Storage: Default Workspaces	
Name	Date
Accudrop template 100um 24psi 39.2KHZ	11/10/2016 11:51 AM
Accudrop template 140um 5.2psi 10.3KHz	11/10/2016 12:28 PM
Accudrop template 200um 3psi 6.1KHz	11/10/2016 12:59 PM
Accudrop template 70um 40psi 81.5KHz	11/10/2016 11:47 AM

Storage: Choose Default

Choose the Experiment template according to the nozzle size.

2. Uncheck "Restore Laser Delay" and "Restore Fludics Setup". Click 'OK'.



3. Place the desired collection device and dummy tubes into the sort chamber and connect the tubes to the ports if cold condition is needed. Place a slide on top of the dummy tubes.





4. Choose the appropriate **Sort Device**. Click '**Sort Ready**' to move the collection device in place.



5. Press '**Plate**' to charge deflection plates. Double click "**Test Streams**" to place a drop of sheath fluid on the slide to see if the position is correct. Adjust the deflection angle of the side streams using the slider and/or adjust the tray position if needed.

- Remove the slide and Click 'Test Streams' to verify if the side streams can enter the dummy tubes.
 Fine tune if needed.
- 7. Click 'Set Home' in the *Tray Control* pane if the tray position has been modified.
- 8. Press 'Plate' to turn off the deflection plates.
- 9. Replace the dummy tubes with the collection tubes that contain enough amount of medium or collection buffer.

10. Create **plots** on the *Worksheet* according to your experimental design.



11. Right click at X and Y axes label and use ADCs to choose your parameters accordingly.



12. Load a tube of you sample, press 'Sample' button beside the sample loading port and click 'Acquire' on the Acquisition Dashboard.



13. Adjust the Sample Offset and set the sample pressure to around 25psi.

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14. Adjust the corresponding PMT voltage using the **slider** on the corresponding axis in a plot or in the *Cytometer* Settings pane.



15. Create **Gates** according to your plot type and experiment design. Make sure the Hierarchy of the gates is correct.

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16. To record FCS file, click the 'Folder' button in the *Recording Settings* pane.



17. Select 'User' folder and create yourself a new folder if you do not have a folder yet.

Select the path where FCS files will be recorded		
🔺 🌽 QC log	*	
36um		
> 퉲 100um		
Cuestroot		
4 🎉 User		
20161109 Goofy		
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3 20161109-carol		
Goofy		
> 🏭 Lena		
🎳 Louise Wong		
BMC_by		
🍌 Xiao Hui	-	
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18. Key in the sample name in the File box.



19. Set the number of events to record (**Event Limit**) and select the appropriate **stopping gate** (e.g. 10.000 events of P3).

		•/·				
Recording Settin	gs					
Recording Keyv	vords					
Folder: User\D)esktop\Q	C log	\100um\			
File: Unstained_001.fcs						
Prefix Unstair	Unstained					
Recording Ru	le					
Event Limit:	10,000					
Time (sec):	Continuo	US				
Stopping Gate:	All Eve	ents				
Storage Gate:	All Eve	ents				
Time Scale (sec):	100					

20. Click '**Record**' on the Acquisition Dashboard.



21. Right click at the corresponding tube position box (**Left/ Right** in the Diagram) in the *Sort Layout* pane. **Assign** the population to be collected.

2	ort Device Sort Mode -	Sort Limit Vunimited 5.00 Sort Report
2	Left	
	O Start O Factor Pacel	0 V 0 10 10 10 10 10 10 10 10 10 10 10 10 1
	● P1 /	■ P2
	Sort: Unlimited	Sort: Unlimited
	Total Events: 0	Total Events 0
Ŧ.	Sort Count: 0	Sort Count: 0
	Sort Rate: 0	Sort Rate: 0
	Abort Count: 0	Abort Count: 0
	Abort Rate: 0	Abort Rate: 0
	Efficiency: 0%	Efficiency: 0%

22. Select the appropriate **Sort Mode.** Set a **Sort Limit** if needed by **unchecked Unlimited** and Key in the target value.

S	ort Device	SIG 197	Sort Mode	Sort Limit	12-1203200
2	Tube Holder - 2 Way S	ort 💌	1.0 Drop Pure	-1981	✓ Unlimited
1000	Left				Right
1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	O Start O Paulo P1 Sort:	Reset]0		© Start P2 Sort:
	Total Events: Sort Count: Sort Rate: Abort Count:	0 0 0 0			Total Eve Sort Cou Sort Rate Abort Co
	Abort Rate: Efficiency:	0 0%			Abort Ra Efficience

- 23. Press '**Plate**' to charge the deflection plates.
- 24. Click 'Sort Ready' and 'Start' in the Sort Layout pane to initiate cell sorting.

Sort Device	Sort Mode	Sort Limit
2 Tube Holder - 2	Way Sort 👻 1.0 Drop Pure 💌	100 V Unlimited
Left		Right
O Start O P1 Sort: Total Events: Sort Count: Sort Rate: Abort Rate: Efficiency:	Value Reset O Unlimited 0 0 0 0 0 0 0 0 0 0 0	Start P2 Sort: Total Even Sort Coun Sort Rate: Abort Cou Abort Cou

25. Click 'Pause' if you want to pause the sort. The Sort Count will continue upon clicking 'Resume'

26. Click '**Stop**' to complete your sort. Click '**Preview**' to view the **Sort Report**. Report can be saved as PDF file if needed.

2 Tube Holder - 2 Way Sort	1.0 Drop Pure 100	✓ Unlimited <u>500</u>	Me De Preview	D
Left		Right	\sim	~
O Start O Prime	Reset 0	O Start	Reset	
9 p1		CQ (2)		
Sort: Unit	mited	Sort:	Unlimited	
Total Events:	0	Total Events:	0	
Sort Count:	0	Sort Count	0	
Sort Rate:	0	Sort Rate:	0	
Abort Count:	0	Abort Count:	0	
Abort Rate:	0	Abort Rate	D	
Efficiency:	0%	Efficiency:	0%	

- 27. Click '**Stop**' on the *Acquisition Dashboard*. Unload your sample and press '**Backflush**'. Allow the flush for at least 5 seconds.
- 28. Continue with other samples or proceed to cleaning procedure.

System Cleaning and Data Saving:

- Load a tube of 3 ml FACS Clean Solution and set the sample pressure to 27.5 psi by adjusting the sample offset. Press 'Sample' and let the solution run for 5 minutes. Unload the tube and Press 'Backflush'.
- 2. Repeat Step 1 with 3 ml FACS Rinse Solution and MilliQ water respectively. Leave the tube of water on the sample port when finish. DO NOT backflush after cleaning with water.



3. Click File > Save > Workspace to save workspace for next time.



Click the icon next to the drag list to **create a New Folder** for yourself. Fill in Workspace Details and click '**OK**' to save.

Save Workspace			
Save V	Vorkspace		
Name	Date	Name: 001 20202	
× 123	11/8/2016 4:34 PM	Description:	
			OK Cancel

4. You may save PDF file by clicking '**PDF symbol**' for each sample and save it in your own folder in the '**User'** folder on **Desktop**.

Works	heet						
3		100%	•	P	N	Go to	•
(<u></u>)(_	-					1	

Data Transfer:

- 1. Double click '**Computer**' icon on desktop.
- 2. Click 'Map Network Drive'. Key in \\192.168.10.202\HKU Portal ID at the Folder. Check 'Connecting using different credentials' and Click 'Finish'.

What n	etwork folder would y	you like to map?	
Specify th	e drive letter for the connec	tion and the folder that y	ou want to connect to:
Drive:	Z:		
Folder	\\192.168.10.202\hku p	ortal	· Browse
	Example: \\server\share	1	
	Reconnect at logon		
	Connect using differ	ent credentials	
	Connect to a Web site th	hat you can use to store y	your documents and pictures-

3. Another window would popup automatically. Click 'Use another account'.

Enter network credentials

Enter your credentials to connect to: 192.168.10.202

Μ	Password]	
	Remember my credentials		
Ρ	Use another account		

4. Enter hkupc2\HKU Portal ID and your portal password. Click 'OK'.

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••••••
 Domain: hkupc2

- 5. Copy and Paste your data from your folder to the server folder.
- 6. Disconnect the Network Drive when finish.

