

CST Quick Guide rev20200401

- 1. Turn on the cytometer and log into your FACSDiVa account as usual.
- All supplies are in white mini-fridge under FACSCalibur workstation.
 Bead dilutions are stable ≥1 week if kept refrigerated and protected from light.
 Use existing tube of CST beads if volume is ≥300ul and age is ≤7 days.
- 3. To make new tube of CST beads (all supplies in white mini fridge):
 - 3.1. Use new 5ml polystyrene FACS tube with cap
 - 3.2. Label tube with "CST", lot#, date: e.g. "CST 80296 3/30/20"
 - 3.3. Vortex blue CST dropper bottle for 2-3s
 - 3.4. Squeeze 1 drop CST bead stock into labeled tube
 - 3.5. Add 600 ul DI H2O and vortex 2-3s
 - 3.6. Immediately return blue CST dropper bottle to

4. In FACSDiVa, go to Cytometer Menu > CST.

BD FACSDiva Software - Ziyi Sang (SBPv2 2-Blue 3-	Red 6-Violet 4-YGr3-UV)			
File Edit View Experiment Populations Worksheet	Cytometer HTS Help			
	Cytometer Details			
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월 🛯 🛠 만 년 🖬 🖉 🖓 -	CST			
	Performance Tracking (LI)			
Name	Cytometer Status Report	Date		
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🕀 🔚 X20 CST 202001-202003	Catalogs	м		
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	Standby			

5. A CST window will pop up, and it will take up to 1 min to connect. The bottom right corner will switch from "Connecting" to "Connected".



- 6. In the new CST app window
 - 6.1. Set Characterize to "Check Performance"
 - 6.2. Lot ID matches blue dropper bottle "80296(RUO
 - 6.3. Check the box for "Load Tube Manually".

		Setup control	
System Summary:	Requires Attention!	Load a tube with beads and click Run button to start setup.	וור
Cytometer Configuration: Lot ID:	SBPv2 2-Blue 3-Red 6-Violet 4-YGr3-UV 80296	Characterize: Oneck Performance	
Cytometer Baseline:	(Reset Target Values) February 04, 2019 09:58 AM Cytometer Baseline is expired. We recommend to re-run the Cytometer Baseline.	Load Tube Manually Plate Type: 95 Well U Bottom	
S Cytometer Performance:	(Failed) March 18, 2020 10:10 AM	Cytometer Configuration: SBPv2 2-Blue 3-Red 6-Violet 4-Y	
X Cytometer Performance Resu	ute: Failed	Select Configuration	
		Setup Beads Lot ID: 80296 (RUO) Product CST Setup Beads	

7. Set fluidics to RUN, LOW, and rotate fine adjustment 5 full turns from either end.

- 8. Vortex bead tube and load on cytometer
- 9. Click the green "Run" button.



9.1. Select "yes" if bead lot has expired. They are stable for many years.

Cytometer Setup and Tracking	Cytometer Setup and Tracking
CST Bead Lot #80296 (RUO) has expired. Do you still want to continue with setup?	Load the CST bead tube (Lot #80296 (RUO)) onto the cytometer. Make sure sheath tank is full and waste tank is empty.
Yes No	OK Cancel

- 10. CST will detect beads and calculate results (can take up to 15min.) If liquid in tube gets too low before the CST completes, click the red "Abort" button, make a new tube of beads as instructed in step 4, and repeat steps 8-9.
- 11. When CST finishes, "Unload the CST bead tube" will pop up.
 - 11.1. Select "OK".
 - 11.2. Unload the tube and put it back to the fridge for the next user.
- 12. A generated PASS/FAIL report will show stats for each channel.
 - 12.1. If fail, PRIME then rerun CST (using beads made today).
 - 12.2. If fail again, email facs@sbpdiscovery.org.

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Cytometer Performance Report									
Cytometer: Cytometer Serial Numl Input Devic Tube Loade Cytometer	Name: ber: e: ed Manually: Configuration:	LSRFortessa X20 H656385091 HTS Yes SBPv2 2-Blue	3-Red 6-Violet	4-YGr3-UV	User: Institution: Software: Date: Cytometer B P/F:	zsan Flow BD F 03/0 aselve: 02/0 Fail	g17 Cytometry ACSDiva 8.0.1 5/2020 09:31 A 4/20:2 09:58 A	M M (Expired)	
Setup Beads Bead Product: CST Setup Beads Part #: 910858 Lot ID: 80296 Expiration Date: 02/29/2020 (Expired) Bead Lot Information: Available									
Laser	Detector	Parameter	Target Value	Actual Target Value	% Difference Target Value	Bright Bead %Robust CV	Mid Bead Median Channel	Mid Bead % Robust CV	
Blue	FSC	FSC	125000	125481	0	0.92	125556	0.92	
Blue	С	SSC	125000	123950	-1	3.06	124769	3.20	
Blue	В	B530	7532	7354	-3	2.01	160	11.36	
Blue	٨	B710	22887	22518	2	2.64	617	12.02	

- 13. Close the window
- 14. 3min bleach +3 min DI water clean, then proceed with your experiment.