



S3e™ Cell Sorter Quick Guide



Checking Fluidics

There are three bulk fluid containers (Figure 1) that may require attention before starting the system. If fluidics need to be refilled, use the quick disconnects to detach the containers from the system and access them.

1. Open the bulk fluids door on the left front of the system.
2. Detach the quick disconnect from the cap assembly by pushing in the metal button and lifting up the quick disconnect until it engages the magnetic holder above it.
3. Slightly lift and pull out the container.
4. Uncap the cap assembly from the container.
5. Refill or change the container.
6. Carefully place the cap assembly into the container and tighten the cap.
7. Place the container into position.
8. Attach the quick disconnect to the cap assembly. An audible click should be heard.

Precautions:

- When handling sheath fluid and deionized (DI) water containers, minimize air exposure to help avoid contamination
- While transferring a cap assembly to a new container, avoid touching the assembly to the outside surfaces of the containers. If it is necessary to set the cap assemblies down, set only on presterilized surfaces
- Treat all waste as biohazardous



Fig. 1. Bulk fluid bottles in the S3e Cell Sorter.

Starting Up the S3e Cell Sorter

Once the containers have been filled/emptied, log into the ProSort™ Software and start up the system.

Note: If the system has been scheduled for an automatic startup, a startup counter will be visible in the log-in window. Please refer to Chapter 9, Automatic Startup, in the S3e Cell Sorter Instruction Manual for startup options.

1. Double click the **ProSort** button (Figure 2) located on the desktop.
2. Log into the software, adding session notes if necessary.
3. Click the **Startup** button (Figure 2) in the Setup and Maintenance tab.
4. Select **Startup Now**, then click the checkmark button in the Startup Settings window (Figure 3).
5. Wait for startup to complete (approximately 20 minutes).



Fig. 2. ProSort and Startup buttons.

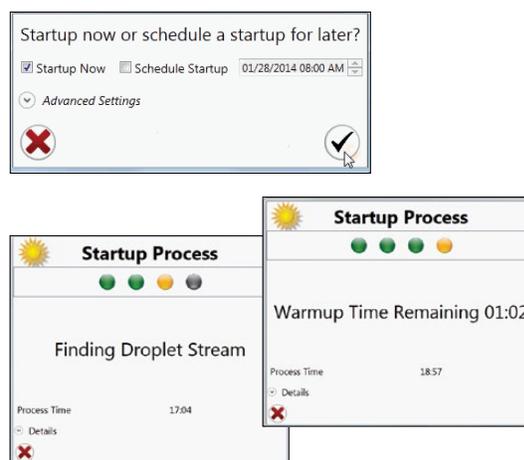


Fig. 3. Windows that appear during startup.

Quality Control

The quality control (QC) procedure must be run every day to ensure optimal system performance. This procedure requires ProLine™ Universal Calibration Beads to adjust alignment, verify QC criteria, set up droplet creation, adjust side streams, calculate drop delay, and monitor droplet break-off.

1. Vortex a bottle of ProLine Universal Calibration Beads and place 10 drops (approximately 0.5 ml) into a 5 ml tube. Do not dilute these particles.
2. Place the tube with beads into the sample input station and move the loading stage into the run position.
3. Click the **Run QC** button (Figure 4) in the Setup and Maintenance tab.
4. Choose the correct bead lot in the Bead Lot dropdown menu in the Event Based Alignment Settings window, then click the checkmark to begin the QC process (Figure 5).

Note: Please refer to section 3.3.1, Editing QC Criteria, in the instruction manual if a new bead lot is being used. It will need to be entered into the system and the QC criteria confirmed. This can be performed only with administrator rights.

5. Once the drop delay calculation has been completed, click the checkmark to close the window (Figure 6).
- A QC report (Figure 7) will appear showing the QC test results.
6. Choose **Save**, **Print**, or **Open QC File**.
 7. When finished, click the checkmark button to close the window.

The system is ready to acquire or sort samples.

Note: Many system functions are disabled until the QC procedure has been completed.



Run QC

Fig. 4. Run QC button.



Fig. 5. Quality Control Process window showing bead lot.

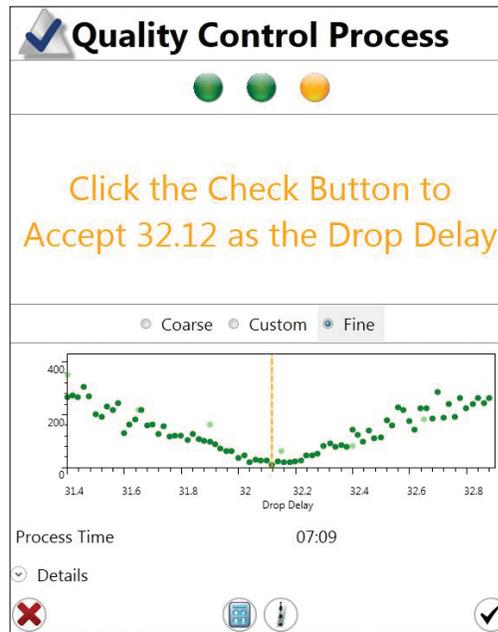


Fig. 6. Quality Control Process window showing calculated drop delay.

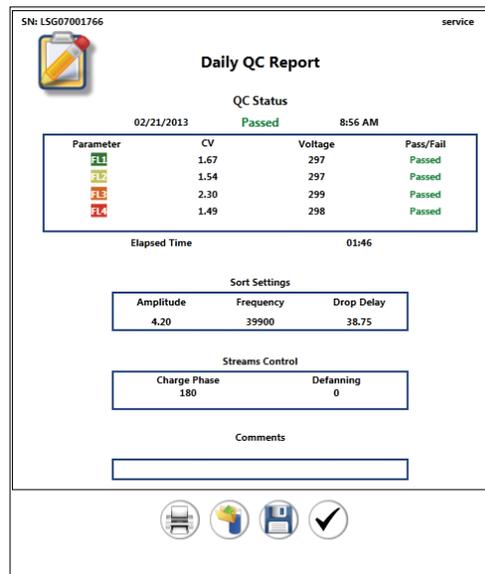


Fig. 7. Daily QC Report window.

Acquisition

Sample acquisition can be performed independent of sorting. As a sample is run through the system, data are gathered and can then be saved in FCS 3.1 format or discarded.

1. Create or open a protocol. At least one histogram or dot plot must be created to continue.
2. Load the sample into the sample input station and move the loading stage into the run position.
3. Detectors can be renamed for each parameter based on the fluorophores or markers used.
4. Check **Cycle Mode** to allow refresh of the data displayed.
5. Define the target event rate, trigger parameter, and threshold value.
6. In the instrument control panel, click the **Start** button to begin sample acquisition (Figure 8).
7. Adjust photomultiplier tube (PMT) voltages to place populations.
8. Adjust the target event rate, trigger parameter, and threshold value.
9. Stop sample acquisition by clicking the **Stop** button.
10. Check and define the event limit. **Gate Limit** can be chosen. **Cycle Mode** can be unchecked at this time.

Optional: Auto Save can be enabled to automatically save the file after the event limit is reached.

11. Re-collect the sample and save the FCS file if Auto Save is not enabled.
12. Unlock the loading stage and remove the sample tube. Move the loading stage into the wash position before the next sample is run.

Note: Cycle Mode can be enabled or the refresh button can be used to refresh the data as adjustments are being made.

When running multicolor experiments, compensation is necessary. In order to use the auto compensation wizard, single stained compensation control samples must be saved in addition to the multicolored samples.

13. Run a single stained compensation control for each parameter.
14. Set the event limit high enough to visualize a positive population, then collect and save the file.
15. Use the auto compensation wizard to set compensation for multicolored samples.

For instructions on compensation methods, please refer to section 5.2, Compensation, in the instruction manual.

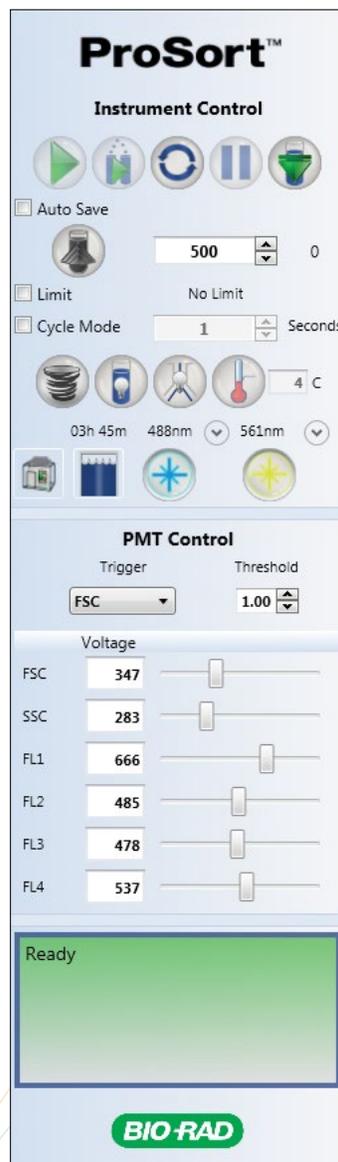


Fig. 8. Start and Stop buttons in the instrument control panel.

Sorting

Most of the sort setup is automatic and is completed during daily QC. When ready to sort, the populations desired must be selected and directed to tubes or wells.

1. Click **File > New Protocol** or **Open Protocol**.
2. Click the **Home** tab, then **Histogram** or **Density** to add plots to the workspace.
3. Adjust temperature control for sample input and collection area if desired.
4. Acquire a small amount of the sample to be sorted.
5. Set sorting regions and gates on plots with the populations of interest.
6. Right click the desired region to be sorted and select the sort direction.
7. Repeat with a second region if two-way sorting is desired.
8. Click the **Sort Logic** button and select a collection vessel in the Sort Logic dropdown menu (Figure 9). Clicking the **Sort Logic** button defaults to the last vessel used (initially set as 5 ml collection tubes).
9. Define the parameters in the Sort Logic window by setting the sort mode (Purity, Enrich, or Single) and event limit for each collection position (Figure 10).
10. Define the sort collection tube volume if you want to track the collected volume to prevent collection overflow (Figure 10).
11. Place the sort collection tubes in the sort collection device at positions 1 and 2, etc. Ensure the 5 ml tubes have at least 0.5 ml of buffer to cushion the sorted cells.
12. Close the sort chamber door and click the **Start Sorting** button in the instrument control panel (Figure 9).
13. The Sort Statistics window (Figure 11) will appear and sorting will begin. It can be reopened by clicking the **Sort Statistics** button in the toolbar.

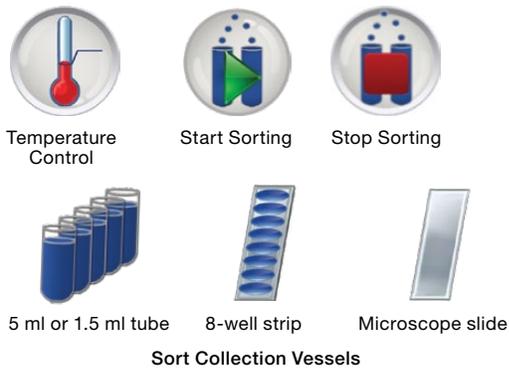


Fig. 9. Temperature Control and various sorting options in the instrument control panel.

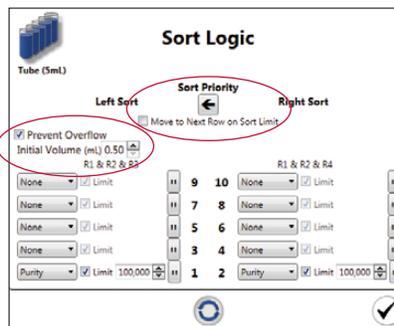


Fig. 10. Sort Logic window displaying various parameters.

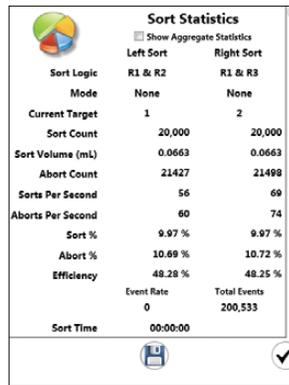


Fig. 11. Sort Statistics window.



Hot Swap

The fluidics can be changed while the system is running a sort or acquisition, which is called a hot swap. Note that new bottles should be prepared before clicking the **Swap Fluidics** button (Figure 12) because the swap time is limited to **3 minutes** before the system will shut down due to low fluid levels.

To perform a hot swap:

1. Click **Swap Fluidics** (Figure 12) in the Setup and Maintenance tab.
2. Click the play button in the Swap Fluidics window (Figure 13).

After clicking the play button, a countdown timer at the bottom of the window will start. There is a limited reserve of fluid in the system and the timer indicates the amount of time left to swap the containers before the system automatically shuts down. Work quickly and carefully during these swapping steps.

3. Open the fluidics door located on the left side of the instrument.
4. Detach the quick disconnect from the cap assembly by pushing the metal button.
5. Lift the quick disconnect until it engages the magnetic holder. This will hold the quick disconnect away from the exchange area.

For sheath fluid or DI water container:

- a. Slightly lift and pull out the empty sheath fluid or DI water container.
- b. Uncap the sheath fluid or DI water cap assembly from the empty container.
- c. Uncap and remove the seal from a new 4 L container.
- d. Carefully place the cap assembly onto the new 4 L container and tighten the cap.
- e. Proceed to step 6.

For waste container:

- a. Slightly lift and pull out the full waste container.
 - b. Uncap the waste cap assembly.
 - c. Carefully empty the waste fluidics container. Avoid contaminating the cap and treat all waste as biohazardous.
 - d. Swap to a new designated waste container or recap the same container.
 - e. Proceed to step 6.
6. Place the container back into position.
 7. Attach the quick disconnect to the cap assembly on the container. An audible click should be heard.
 8. Click the play button in the window again to finish the swapping procedure.



Swap Fluidics

Fig. 12. Swap Fluidics button.

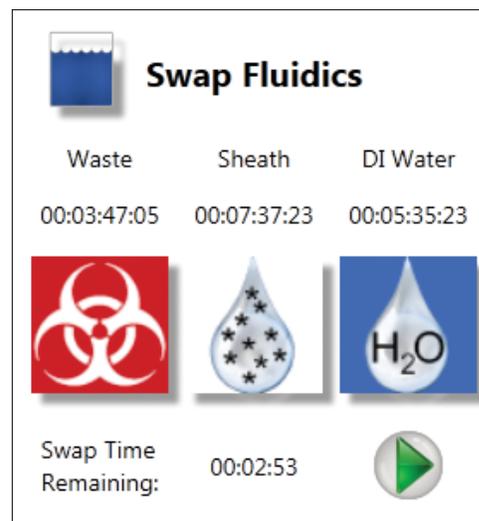


Fig. 13. Swap Fluidics window.



Shutting Down the S3e Cell Sorter

1. Ensure that the sample loading stage is in the wash position.
2. Click the **Shutdown** button (Figure 14) in the Setup and Maintenance window toolbar.
3. Select **Yes** to confirm that the system should be shut down at this time.
4. Select **Yes** to continue without saving protocols, or abort the shutdown procedure to save protocols.
5. If using the automatic startup option, choose **Yes** in the Automatic Startup window, then select the start date and time (Figure 15).
6. If running a clean procedure during shutdown, choose **Yes** in the Automatic Startup window.
7. When prompted (Figure 16), load a tube of cleaning solution and move the loading stage into the run position.
8. When prompted, move the loading stage into the wash position.

At this time the system is fully shut down. The software will return to the opening log-in screen and can be left in standby mode until the next use.



Shutdown

Fig. 14. Shutdown button.

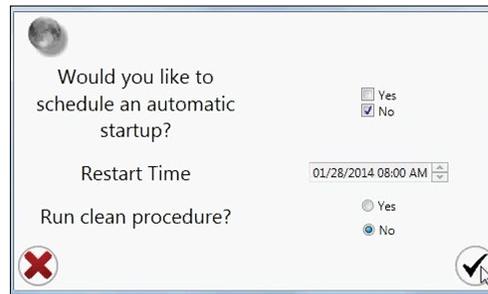


Fig. 15. Automatic Startup window.

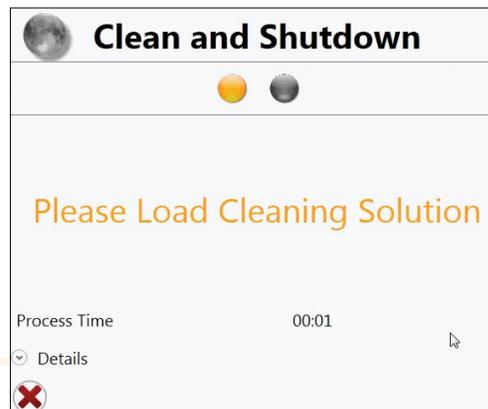


Fig. 16. Clean and Shutdown window.



Bio-Rad Technical Support

For help and technical advice, please contact the Bio-Rad Technical Support department. In the United States, the Technical Support department is open Monday–Friday, 5:00 AM – 5:00 PM, Pacific time.

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Ordering Information

Catalog # Description

Instrumentation

145-1005	S3e Cell Sorter (488 nm)
145-1006	S3e Cell Sorter (488/561 nm)
145-1008	S3e Cell Sorter (488/640 nm)
145-1078	S3e Biosafety System Class I
145-1029	S3e Cell Sorter (488 nm) with S3e Biosafety System Class I
145-1030	S3e Cell Sorter (488/561 nm) with S3e Biosafety System Class I
145-1032	S3e Cell Sorter (488/640 nm) with S3e Biosafety System Class I

Consumables

145-1082	ProFlow™ Sort Grade 8x Sheath Fluid, 5 x 4 L
145-1083	ProFlow Sort Grade Water, 5 x 4 L
145-1085	ProLine Rainbow Beads, 1 x 5 ml
145-1086	ProLine Universal Calibration Beads, 3 x 5 ml

Accessories

145-1065	S3e Accessory Kit
145-1084	S3e Fluidic Container, 3 x 4 L
144-1001	S3e Sheath Cap Assembly, white
144-1002	S3e DI Water Cap Assembly, blue
144-1003	S3e Waste Cap Assembly, red
144-1005	S3e Filter Block A, empty with 2 dichroic filter holders
144-1006	S3e Filter Block B, empty with 2 dichroic filter holders
144-1007	S3e Individual Filter Holder, black, empty
144-1008	Neutral Density Filter, 1.0
144-1009	S3e Nozzle Tip, 100 µm
144-1010	S3e Nozzle O-Ring with Alignment Disk, 2-pack
144-1011	S3e Collection Adaptor Set
144-1012	Hex Driver, 2 mm
144-1013	S3e Individual Dichroic Filter Holder
145-1076	S3e Power Conditioner, international
145-1077	S3e Power Conditioner, U.S.

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