invitrogen

Bigfoot Cell Sorter Quick Start Guide

DM00109 Rev. B Approved: 12 July 2021

Refer to the Bigfoot Instruction Manual in the Help section of Sasquatch Software (SQ Software) for detailed instructions on how to use the Bigfoot instrument and software.

Instrument Startup

1. Open SQ Software and log in.
2. Check system status. Ensure that the instrument is powered on and connected to the operating computer. Fill the bulk fluids containers and empty the waste containers if necessary. Note that the fluid containers can be exchanged during operation.
3. Click the **Startup** icon.

Quality Control

The quality control procedure should be run every day to ensure optimal system performance. This procedure verifies the filter set and QC criteria, adjusts alignment, adjusts PMT voltages, tests the event rate, sets up droplets and streams, calculates drop delay and stores the results. A bottle of Bigfoot QC Particles must always be in place in the bead bottle position of the sample loader and will last for several months depending on usage.

1. From the **Home** screen, click **Quick Run**.


Quick Run

2. In the **Flex Controls** on the right-side panel, click **Setup**.


Setup

3. Click **1:QC Setup** and then the **Bead Alignment** icon.


Bead Alignment

4. After bead QC is complete, click **2:Droplet Setup** and then the **Maintain Droplets** icon.


Maintain Droplets

5. After droplets are maintained, click **3:Streams Setup** and then the **Setup Streams** icon.


Setup Streams

6. After streams setup is complete, click the **Adjust Drop Delay Deflection** icon.


Adjust Drop Delay Deflection

7. After deflection setup is complete, click **4:Drop Delay Setup**. Set the drop delay streams and then click the **Auto Drop Delay** icon.


Adjust Drop Delay

8. After the drop delay calculation is complete, the system is ready to run. The QC and droplet icons on the top of the control panel will appear white.


QC Readv

ThermoFisher SCIENTIFIC

Home Screen

After logging into the software, you have the following choices:

- **New Sort** –Experiment Builder provides the steps to set up a new sort experiment.
- **Quick Run** – Enables all parameters and brings you to the workspace to set up and run experiments manually. The QC protocol is accessed through the Quick Run screen.
- **Resume** – Resumes the last protocol that was used in the workspace.
- **Open Protocol** – Opens a file manager and allows you to select an existing experiment.

To set up a **New Sort**

New Sort

1. Click the **New Sort** button.
2. Assign the experiment a name in the **Name** screen and click the **Next** arrow at the bottom of the screen.
3. Select compensation or spectral unmixing if applicable.
4. In the **Fluorophores** screen, double-click fluorophore names to add them to the experiment and select sample control options if desired. Click the **Next** arrow.
5. Place samples into the loader in the order shown in the samples panel.
6. In the workspace, run the negative sample and adjust settings as desired.
7. Record control samples and use the auto-compensation wizard if selected to remove spillover.
8. Set up plots in the primary group for sorting. Create regions and assign them as sort regions by right clicking in the region.
9. From the **Flex Controls**, click the **Sort Logic** icon and assign sort directions, modes, and limits.
10. Run a little bit of the sample to be sorted and adjust regions/gates as needed.
11. Click the **Start Sort** button and review the preview page. Ensure the correct media is placed in the sort output area and the streams are deflected correctly. Press the Check Deflection button to confirm.
12. Click Start sort to begin sorting.

Sort Logic

To set up a **Quick Run**:

Quick Run

1. Click **Quick Run** in the main screen. The workspace opens.
2. Configure the workspace by manually creating plots and regions.
3. Acquire samples and set up sorting if desired.

Acquiring Data from a Loader Position

Acquire from the loader if you are running one or only a few samples.

Run

1. After setting up the workspace, select the sample to be run and verify the position in the sample list. Set the **Event Limit** if desired.
2. Place the sample to be acquired in the assigned position.
3. Click the **Run** button to acquire the sample.
4. Adjust settings and regions.
5. When you are ready to save data, click the **Record** button.
6. Acquisition will stop automatically if an event limit was set. If not, click the **Stop** button when you are finished.

Record

Analysis

After acquiring samples, you can analyze the data in the workspace.

To analyze data immediately after acquisition that is still present in the workspace:

1. Click on the play button next to the desired run in the sample list. Data will be loaded into the selected group.

To analyze data that has been saved but is no longer present in the workspace:

1. Click the **+** button in the **FCS Files** panel.
2. Select the desired FCS files.
3. Once the FCS files are loaded, assign a group from the drop-down list, and click the **Play** button to load the data into the selected plots.
4. Create plots and regions as necessary for analysis.

Shutdown

1. Click the **Shutdown** icon.
2. Schedule automatic startup if desired.
3. The system automatically depressurizes the fluidics, rinses the system with cleaner, turns off all the lasers, puts the nozzle in the storage location and enters a shutdown/low airflow state until **Startup** is initiated.

System Support

For service and support, please contact the manufacturer at:
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Fort Collins, CO 80524 USA
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http://www.thermofisher.com