Abort Count Quantification Protocol

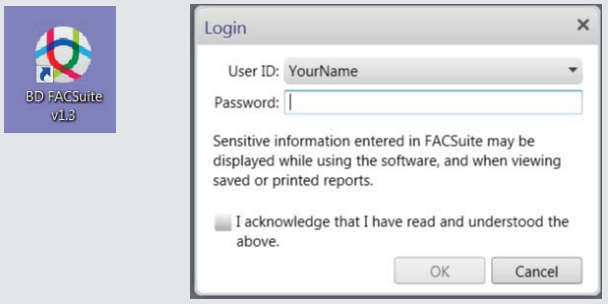
This procedure applies to the BD FACSLyric™ flow cytometer

Materials Needed

- BD Trucount™ Tubes
- BD FACSFlow™ Sheath fluid
- BD FACSClean solution or 10% bleach (if necessary)

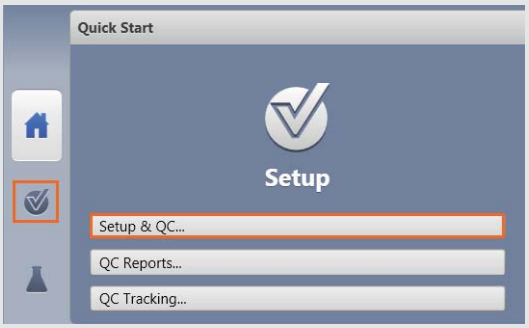
Procedure

Open BD FACSuite software and log in.



Perform Daily Performance Check

1. Prepare a tube of BD CS&T Beads according to the directions in the technical data sheet.
2. On the navigation bar, click **Setup & QC**.
   - The **Setup & QC** workspace opens



3 In the **Setup & QC Options** panel, verify that the **Performance QC** task is selected.

4 Verify that the correct CS&T Bead lot ID is selected.

5 In the **Setup & QC Options** panel click **Start**.

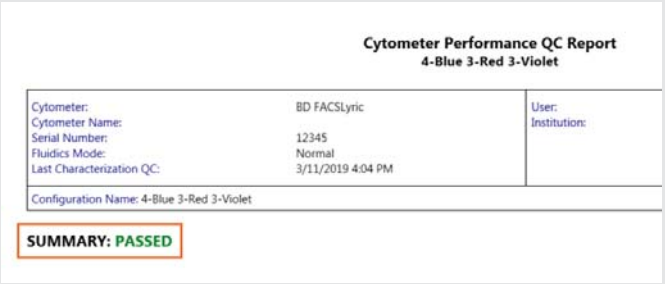
- The Load Tube dialog opens.

6 Load the tube of BD CS&T Beads onto the manual tube port.

- The system detects the tube, and the setup tasks begin.
- When all tasks are complete, a dialog opens and indicates whether the task completed successfully.

7 Click **Yes** to view the Performance QC report and verify that the **SUMMARY** reads **PASSED**.

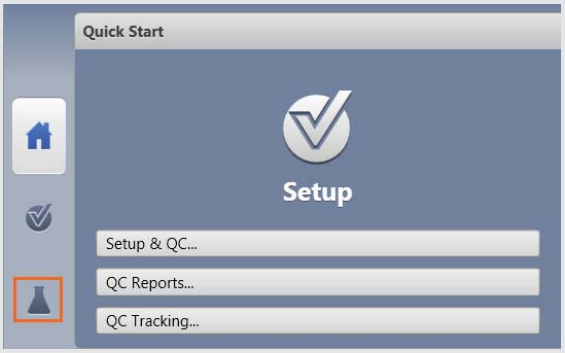
8 Unload the tube.



Create an experiment

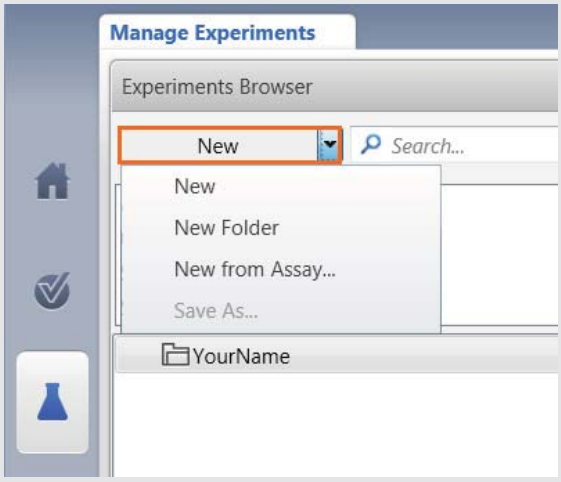
1 On the navigation bar, click **Experiments**.

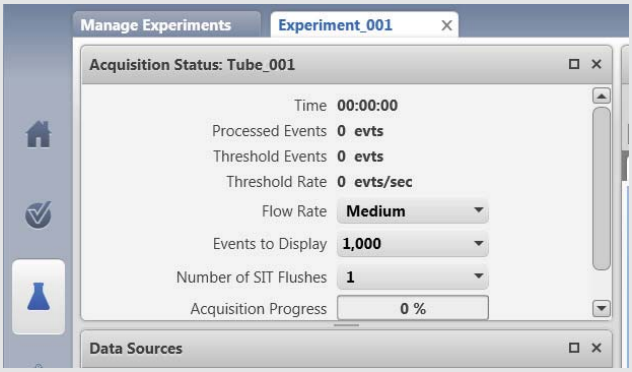
- The **Manage Experiments** tab opens in the Experiments workspace.



2 In the **Experiments Browser** panel, click **New**.

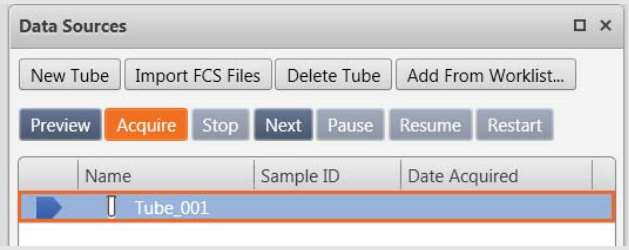
- A new experiment opens. The new experiment name and creation date are displayed in the **Experiments Browser**, and a new tab opens in the Experiments workspace.
- (You may consider renaming the Experiment as Instrument Serial Number)



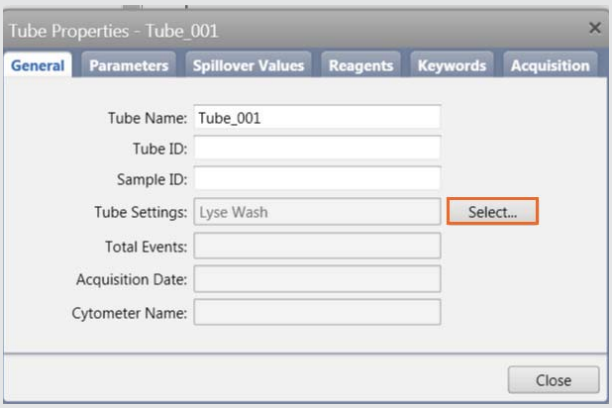


3 In the **Data Sources** panel, double click on **Tube_001**.

- (You may consider renaming the Tube as <TruC_Lot number of the TruC>).



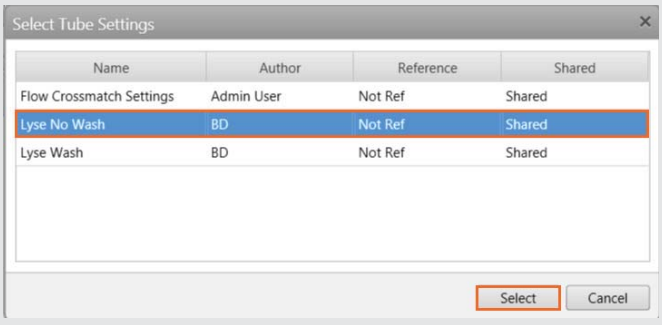
4 The **Tube Properties** dialog box opens.



5 On the **General** tab, click on **Select** to the right of the **Tube Settings**.

6 In the **Select Tube Settings** dialog panel, choose **Lyse No Wash**

7 Click on **Select**.

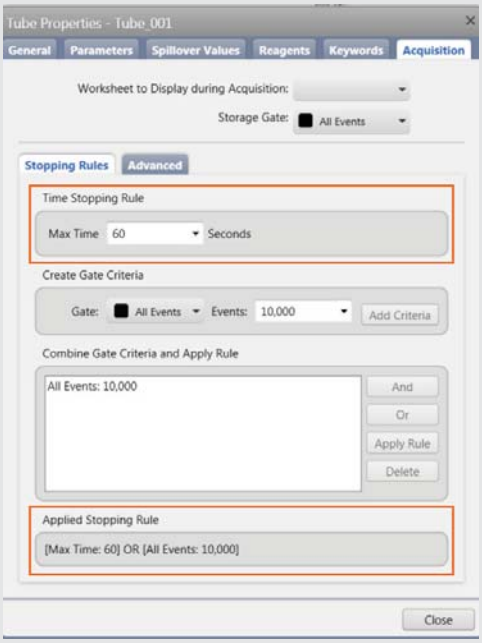


8 In the **Tube Properties** Dialog box, click on the **Acquisition** tab.

9 Change the **Time Stopping Rule** by entering **60 seconds** as the **Max Time** and press Enter. Verify the **Applied Stopping Rule**.

10 Click Close.

11 **No additional plots or gates need to be created.**



Preparing BD Trucount Tubes

Store BD Trucount tubes in their original foil pouch at 2°C–25°C.

- Open the pouch only after it has reached room temperature, if stored in the refrigerator, and carefully reseal the pouch immediately after removing a tube.
- Examine the desiccant each time you open the pouch.
- If the desiccant has turned from blue to lavender, discard the remaining tubes.
- Use the tube within 1 hour after removal from the pouch.
- Examine the BD TruCount tube and ensure that the pellet is below the retainer and intact as shown.

1 Pipette 500 µL of sheath fluid into the BD Trucount tube.

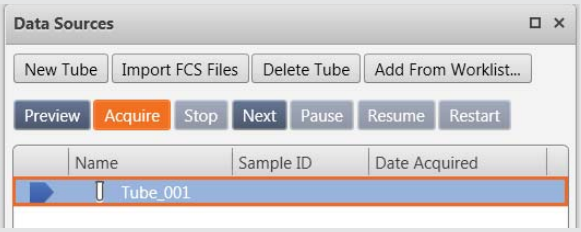
NOTE: We recommend the use of calibrated pipettes. Do not estimate the volume when adding sheath fluid. The addition of a precise volume is critical to determining an accurate count.

2 Vortex the tube gently to mix.

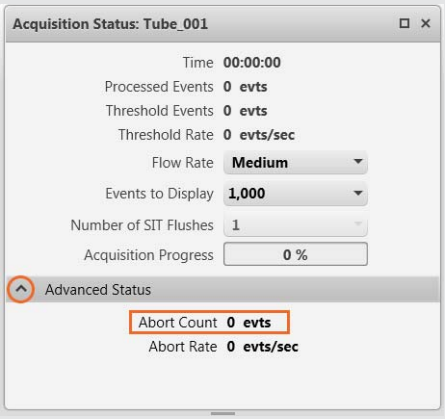
Acquiring the BD Trucount tube

1 In the **Data Sources** panel, set the run pointer to Tube_001.

2 Install the BD Trucount tube on the manual tube port.



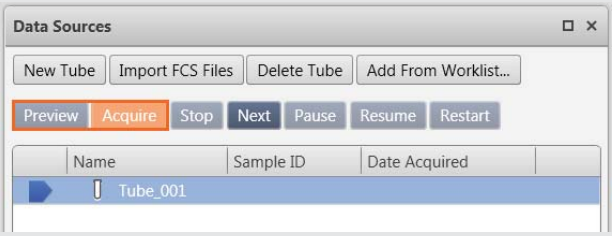
3 In the **Acquisition Status** panel, click the arrow to the left of **Advanced Status** to display additional information.



4 Click **Preview** in the **Data Sources** panel.

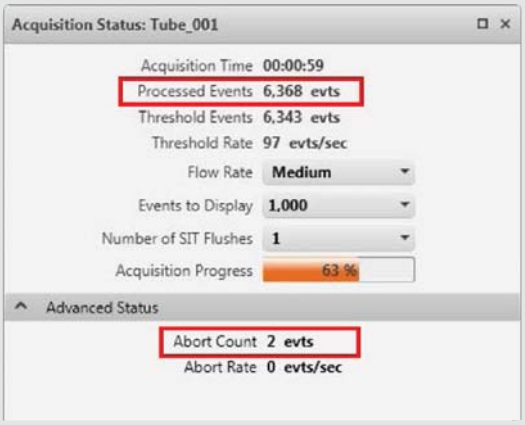
- Preview for at least **17** seconds before acquiring data. This delay is necessary to ensure a proper count.

5 Click **Acquire** and wait for the acquisition to complete.



6 Record the Abort Count and Processed Events as shown in the Acquisition Status panel

7 Remove Trucount tube from Manual tube port and install a tube of DI.



- **<u>If the percentage of aborted events is less than 1.0%, your instrument is not impacted. Please follow the instructions at the end of ths document to report your results.</u>**
- **<u>If the percentage of aborted events is greater than or equal to 1.0%, perform troubleshooting as stated below.</u>**

- To perform this calculation, divide the Abort Count by the Processed Events then multiply by 100 to determine the percentage of aborted events.

$$\frac{\text{Abort Count}}{\text{Processed Events}} \times 100 =$$

If the value is < 1.0%, you are not impacted

If the value is ≥ 1.0%, perform troubleshooting procedure

Example: Less than 0% aborted events

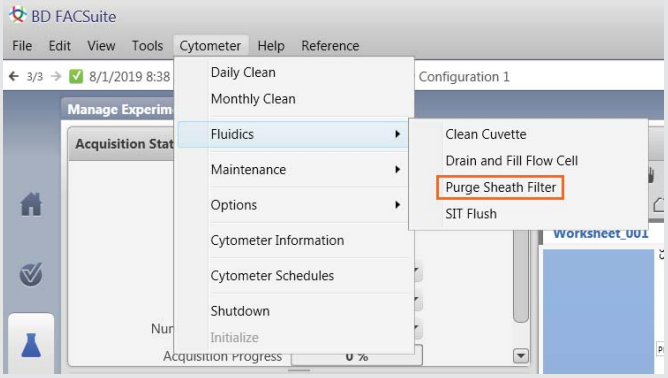
$$\frac{2}{6368} \times 100 = 0.03\%$$

Example: Greater than 4.4% aborted events

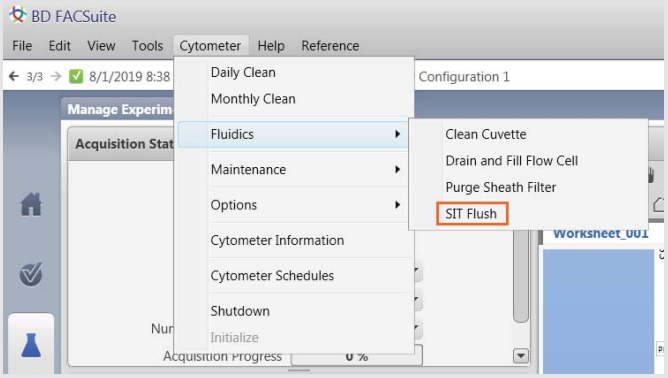
$$\frac{230}{5234} \times 100 = 4.39\%$$

Troubleshooting procedures:

1 Go to **Cytometer > Fluidics > Purge Sheath Filter** to remove air from the sheath filter. Repeat as needed.

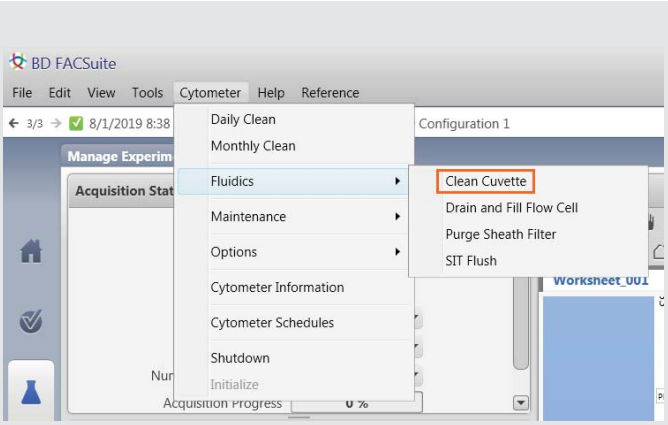


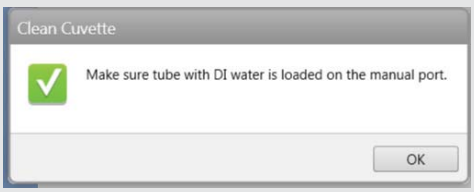
2 Go to **Cytometer > Fluidics > SIT flush** to flush the SIT. Repeat as needed.



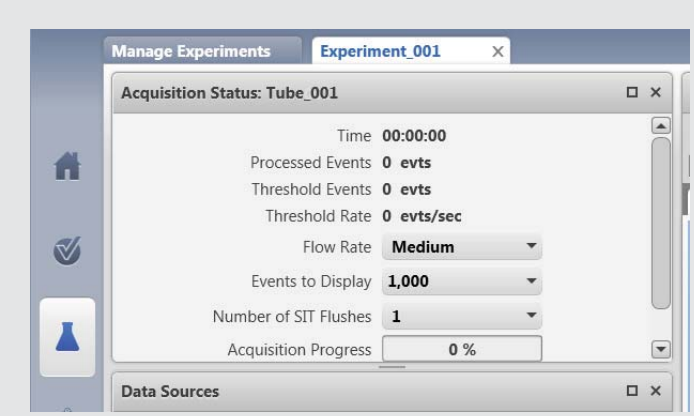
3 Go to **Cytometer > Fluidics > Clean Cuvette** clean the cuvette.

- Install a tube containing 3 mL of 10% FACSClean or 10% bleach on the manual tube port and click **OK**.
- When the process is complete, the dialogue box closes automatically.
- Allow the 10% bleach to remain in cuvette for a several minutes. Remove the tube.
- Go to **Cytometer menu > Fluidics commands > Clean Cuvette**.
- Install a tube containing 3 mL of DI water on the manual tube port and click **OK**.
- When the process is complete, the dialogue box closes automatically.
- Allow the water to remain in cuvette for a several minutes. Remove the tube.



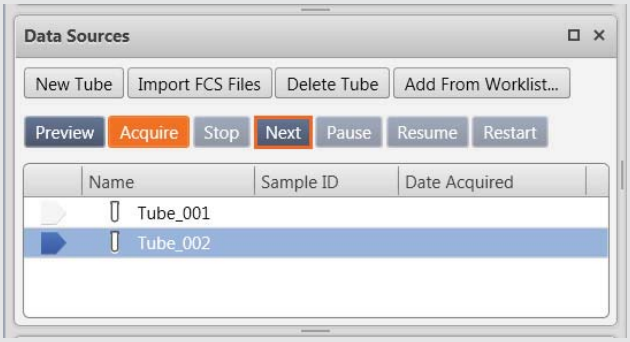


4 Navigate back to the Experiment workspace.



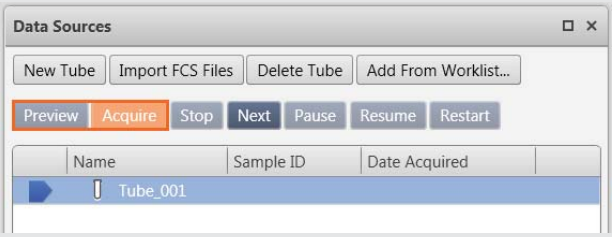
5 Vortex the BD Trucount tube.

6 In the Experiment, click on **Next** to add an additional tube.



7 Click **Preview** in the **Data Sources** panel.
- Preview for at least 17 seconds before acquiring data. This delay is necessary to ensure a proper count.

8 Click Acquire and wait for the acquisition to complete.
- Capture the Abort Count and Processed Events using a mobile phone or Snipping tool on the computer.



If the percentage aborted events is less than 1.0%, no follow-up activities are required for your instrument

a. Email the customer reply form to techsupport@bd.com

If the percentage aborted events is greater than or equal to 1.0%, follow-up activities are required for your instrument

b. Email the customer reply form to techsupport@bd.com or telephone 0800 917 8776, select option 1, then 3.

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BD Life Sciences, San Jose, CA, 95131, USA

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