



Title: Operation and Standardization of the FACSCalibur Flow Cytometer

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1.0 Purpose

This procedure describes the operation and calibration of the FACSCalibur Flow Cytometer.

2.0 Scope

This procedure applies to Biopharmaceutical Development Program (BOP) personnel operating the FACSCalibur Flow Cytometer..

3.0 Authority and Responsibility

- 3.1 The Director, Process Analytics/Quality Control (PA/QC) has the authority to define this procedure.
- 3.2 The Director, PA/QC or designee is responsible for training laboratory personnel and documenting this training to Biopharmaceutical Quality Assurance (BOA).
- 3.3 PA/QC personnel are responsible for the performance of this procedure.

3.4 PA/QC are responsible for reviewing the data and documentation of the results of this procedure.

3.5 BQA is responsible for quality oversight of this procedure.

4.0 Equipment and Supplies

4.1 FACSCalibur, Serial # E4219 and MEF #79100, BD Biosciences

4.2 Apple MacIntosh computer

4.3 Software: FACSCComp Version 4.2; CellQuest

4.4 12 x 75 mm tube, BDP PN 20396 or BDP approved equivalent

4.5 Bleach, BDP PN 10579 or BDP approved equivalent

4.6 FACSFlow sheath fluid, BDP PN 30535 or BDP approved equivalent

4.7 CaliBRITE 3 beads (containing Unlabeled, FITC-, PE-, PerCP-labeled beads), BDP PN 30681 or BDP approved equivalent

4.8 CaliBRITE APC beads, BDP PN 30680 or BDP approved equivalent

5.0 Operation

5.1 FACSCalibur Daily Startup

5.1.1 Turn on the **cytometer**. Record in the equipment logbook the activity, date, time and technician's name/initial (refer to **SOP 21531 - Equipment Logs**).

5.1.2 Turn on the **computer**.

5.1.3 Fill the sheath tank with FACSFlow sheath fluid (4.6) and verify the following.

5.1.3.1 Tank is full (~ 3L).

5.1.3.2 Cap is tight.

5.1.3.3 Tank is pressurized.

5.1.3.4 Tubing is not kinked.

5.1.4 Empty the waste tank, add ~400mL of straight bleach (4.5) into the waste tank and verify that the tubing is not kinked.

5.1.5 Purge bubbles from the sheath filter.

5.1.5.1 Allow the cytometer to warm up for 5 minutes.

5.1.5.2 Place a tube containing 1 mL of FACSFlow sheath fluid on the Sample Injection Port (SIP) of the cytometer.

5.1.6 Push the **RUN** button on the cytometer. **If setup is fine and the instrument is ready to make runs, the RUN button lights up green; otherwise, it remains yellow.**

5.2 Instrument Quality Control-Running FACSCComp

5.2.1 Follow steps 5.1.1 to 5.1.5 for starting up the cytometer.

5.2.2 Turn on the computer.

5.2.3 Make up the tubes of beads needed to perform the FACSCComp run (Four colors).

- 5.2.3.1 **Tube #1:** Add one or two drops of **Unlabeled** and **APC-labeled CabliBRITE beads** into the tube containing 1 mL of sheath fluid.
- 5.2.3.2 **Tube #2:** Add one or two drops of **Unlabeled, FITC-, PE-, PerCP-, and APC-labeled CabliBRITE beads** into the tube containing 3 mL of sheath fluid.
- 5.2.4 Launch FACSComp from the computer menu.
- 5.2.5 Enter Operator, Institution and Director Information at the sign-in view. Verify that the Cytometer Model and serial number are correct and click **Accept**.
- 5.2.6 The **Setup** view appears, select **Lyse/Wash** under Assay Selection.
- 5.2.7 Enter the CaliBRITE lot IDs. The bead lot ID, consisting of five numbers and one letter (without space), is found on the kit flyer included with the CabliBRITE beads. Enter them before continuing.
- 5.2.8 Follow instructions displayed in the message box underneath the icon bar and Run FACSComp.
- 5.2.9 PMT Adjustment
- 5.2.9.1 Gently mix the unlabeled (or unlabeled and APC) bead Tube #1 and install Tube#1 on the SIP of the cytometer. Sample flow is set to **HI** and the cytometer is set to **RUN**.
- 5.2.9.2 Click Start to begin PMT adjustment. After PMTs are set, the message **PMTs Set Successfully** appears. The software counts down for 5 seconds before it automatically advances to the time-delay calibration.
- 5.2.10 Time-Delay Calibration (for 4-color setup)
- 5.2.10.1 FACSComp performs a time-delay calibration immediately after PMTs are set.
- 5.2.10.2 The unlabeled and APC beads Tube #1 is left on the cytometer until time-delay calibration is complete. A message appears indicating time-delay calibration is being performed.
- Performing Time Delay Calibration
- 5.2.10.3 If calibration is successful, then FACSComp displays the message.
- Time Delay Calibration was successful
- NOTE:** If the calibration was unsuccessful, select either re-entry time-delay calibration or repeat initial PMT adjustment. Should the calibration remain unsuccessful, do not use the instrument. Contact the area Supervisor for instructions.
- 5.2.11 Compensation Adjustment
- 5.2.11.1 After PMTs and time-delay calibration are successfully complete, FACSComp proceeds to Compensation view.
- 5.2.11.2 Remove the unlabeled (or unlabeled and APC) beads Tube #1 from SIP of the cytometer.

5.2.11.3 Gently mix the mixed beads in Tube #2 and install Tube #2 on the SIP.

5.2.11.4 Click Start to begin compensation adjustment. FACSCComp adjusts appropriate compensation until target - mean difference for FL4 is achieved. When compensation is set, **Compensation Set Successfully** message appears. The software counts down for 5 seconds before advancing to the Sensitivity view.

NOTE: FACSCComp will go into a manual mode if 1) Flow rate is < 400 beads/sec, 2) Automatic compensation takes longer than 75 seconds, 3) Compensation is > 50%. To activate manual mode, click the Manual button at the bottom of the screen.

5.2.12 Sensitivity Tests

5.2.12.1 After compensation is set, FACSCComp advances to the Sensitivity Test view

5.2.12.2 The mixed bead tube is left on the cytometer and the Fluorescence Sensitivity Test acquisition display appears. It consists of:

- Fluorescence Sensitivity Test
- Side Scatter Sensitivity Test
- Forward Scatter Sensitivity Test. FACSCComp acquires 10,000 events and calculates FL1, FL2, FL3 fluorescence separation between the unlabeled and labeled (FITC, PE, and PerCP) beads.

For FL4 the software measures the uncompensated signal separation between the PerCP and APC beads in the FL4 channel. It next proceeds to the Side Scatter Sensitivity and then to the Forward Scatter Sensitivity. FACSCComp calculates the separation between noise and side scatter signal (Side Scatter Sensitivity) and noise and forward scatter signal (Forward Scatter Sensitivity) of the beads after acquiring 10000 events. When this is complete, the results are calculated, and the Calib File is saved in the Instrument Settings Files Folder in the BD Files folder.

5.2.12.3 Remove the beads tube and install a tube of DI water when the FACSCComp run is completed. Place the instrument in **STANDBY**.

5.2.12.4 Select Print from the File menu to print the Summary Report. The Summary Report contains histograms and the separation for each parameter, the current FACSCComp instrument settings, results of time-delay calibration (if 4-color setup was performed), and the Sensitivity Test results that is either Pass or Fail. If the channel separation is greater than or equal to the minimum channel separation, the parameter will pass the Sensitivity Test. **For the QC test, attach the calibration information with the QC test report.**

5.2.12.5 Quit FACSCComp software.

NOTE: If any of the tests failed, re-run the test. Should the calibration remain unsuccessful, do not use the instrument. Contact the area Supervisor for instructions.

5.3 CellQuest

- 5.3.1 Turn on the cytometer prior to turning on the computer to ensure proper initialization between the cytometer and the computer.
- 5.3.2 Launch CellQuest software from the computer menu.
- 5.3.3 Connect to Cytometer.
 - 5.3.3.1 Choose Connect to Cytometer (**æB**) from the Acquire menu.
 - 5.3.3.2 Choose Detectors/Amps (**æ1**) from the Cytometer menu.
 - 5.3.3.3 Choose Threshold (**æ2**) from the Cytometer menu.
 - 5.3.3.4 Choose Compensation (**æ3**) from the Cytometer menu.
- 5.3.4 Restore Calib Settings to the Instrument.
 - 5.3.4.1 Choose **Instrument Settings** from the Cytometer menu.
 - 5.3.4.2 The Instrument Setting window appears. Click **Open**.
 - 5.3.4.3 Click in the pop-up menu and choose the Macintosh hard disk.
 - 5.3.4.4 Click BD Files folder; then click open.
 - 5.3.4.5 Scroll to the Instrument Settings Files folder using the slider; then double-click the **Instrument Settings Files folder**.
 - 5.3.4.6 Click the **Calib File**.
 - 5.3.4.7 Click **Open**.
 - 5.3.4.8 Click **Set** in the Instrument Settings window.
 - 5.3.4.9 Click **Done**.
- 5.3.5 Prepare the screen for sample optimization.
 - 5.3.5.1 Expand the experiment window to full size by clicking the zoom box in the upper right-hand corner of the window.
 - 5.3.5.2 Select the **Dot-Plot** tool from the tool palette.
 - 5.3.5.3 Click in a clear area of the Experimental window and drag diagonally until the plot outline is the desired size.
 - 5.3.5.4 Click and hold the plot Source box to open the pop-up menu and choose **Acquisition**.
 - 5.3.5.5 The X and Y parameters default to **FSC** and **SSC**.
 - 5.3.5.6** Click the Multicolor Gating radio button in the colors box. Click **OK**.
 - 5.3.5.7 Repeat Steps 5.3.5.1 through 5.3.5.5 to make the FL1 vs FL2 plot, FL2 vs. FL3 plot, and FL3 vs. FL4 plot, if necessary.
- 5.3.6 Optimize FSC, SSC, FL1, FL2, and FL3 Detectors (Refer to the **Manual: Pages 5-20 to 5-27**).
 - 5.3.6.1 Place the flow cytometer in **RUN** mode.
 - 5.3.6.2 Place the isotype control tube on the Sample Injection Port (SIP).
 - 5.3.6.3 Adjust the **FSC and SSC Detectors**. The Mode should be set to **Lin**.
 - 5.3.6.4 Adjust the **FSC Threshold Setting**.

- 5.3.6.5 If necessary, Set a **Region around the cell population of interest**.
- 5.3.6.6 Adjust the **FL1, FL2, and FL3 Detectors**. The Mode should be set to **Log**.
- 5.3.6.7 **Do not adjust the FL4 PMT Voltage. Leave the voltage value set by FACSComp.**
- 5.3.6.8 Remove the subclass control tube from SIP.
- 5.3.7 Adjust Fluorescence Compensation (Refer to the **Manual: Pages 5-27 to 5-31**).
 - 5.3.7.1 Place the positive control tube on the Sample Injection Port (SIP).
 - 5.3.7.2 For example, adjust the **FL2-%FL1** by clicking the up and down arrow to place the **FITC+** population in the lower-right quadrant.
 - 5.3.7.3 Remove the positive control tube from the SIP and install a tube of DI water on the SIP.
 - 5.3.7.4 If necessary, adjust FL1-%FL2, FL3-%FL2, FL2-%FL3, FL4-%FL3, or FL3-%FL4 using appropriate positive control tubes.
 - 5.3.7.5 Click Pause and then Abort in the Acquisition Control window.
 - 5.3.7.6 Place the flow cytometer in **STANDBY** mode.
- 5.3.8 Pre-Acquisition Software setup.
 - 5.3.8.1 Set up the Acquisition and Storage window.
 - 5.3.8.1.1 Choose **Acquisition and Storage** from the Acquire menu.
 - 5.3.8.1.2 Leave the defaults Accept and All in the Acquisition Gate pop-up menus.
 - 5.3.8.1.3 Leave the **defaults Event Count, 10000**, and **All** in the Collection Criteria pop-up menus.
 - 5.3.8.1.4 Leave the **default All** in the Storage Gate pop-up menu.
 - 5.3.8.1.5 Leave the **default 1024** for Resolution.
 - 5.3.8.1.6 Click **OK** in the Acquisition and Storage window.
 - 5.3.8.2 Edit Reagent Panels (Refer to the Manual Pages **5-38 to 5-41**).
 - 5.3.8.3 Set up the Parameter Description window.
 - 5.3.8.3.1 Choose Parameter Description from the Acquire menu.
 - 5.3.8.3.2 Click the **Folder** button.
 - 5.3.8.3.3 Click the destination folder pop-up menu.
 - 5.3.8.3.4 Choose the hard drive name and your folder (e.g., Desktop/Ch14.18).
 - 5.3.8.3.5 Click the **File** button.
 - 5.3.8.3.6 Type a file name in the highlighted Custom Prefix field of the File Name Editor window (e.g., GD2-040806).
 - 5.3.8.3.7 Choose File Count from the File Name Suffix pop-up menu.

- 5.3.8.3.8 Make sure **1** is entered in the File Count: field.
- 5.3.8.3.9 Click **OK**.
- 5.3.8.3.10 Type in information user wants to save with the data files in the Patient ID, Sample ID, and Comments boxes.
- 5.3.8.3.11 Click the **panel checkbox**.
- 5.3.8.3.12 Choose the **reagent panel** that was created in step 5.3.8.2 in the panel pop-up menu.

5.3.9 Acquisition of Data

- 5.3.9.1 Choose Counters from the Acquire menu.
- 5.3.9.2 Move the window to a clear area of the screen.
- 5.3.9.3 **Deselect the Setup checkbox** in the Acquisition Control window.
- 5.3.9.4 Place the flow cytometer in **RUN** mode.
- 5.3.9.5 Install the first tube on the SIP. Immediately place the support arm under the sample tube.
- 5.3.9.6 **Wait** approximately **5 seconds** before clicking Acquire.
- 5.3.9.7 Click **Acquire**.
- 5.3.9.8 Remove the tube **after the beep**.
- 5.3.9.9 Place the next tube on the SIP.
- 5.3.9.10 After waiting approximately 5 seconds, click **Acquire** and remove the tube after the beep.
- 5.3.9.11 Repeat steps 5.3.9.9 and 5.3.9.10 for the remaining tubes in the panel.
- 5.3.9.12 **Select the Setup checkbox**.
- 5.3.9.13 Click and close boxes of the Acquisition Control window and the Counters window when all the tubes have been acquired.
- 5.3.9.14 Disconnect from the cytometer.
- 5.3.9.15 Place the flow cytometer in **STANDBY** mode.

5.4 FACSCalibur Daily Shutdown

- 5.4.1 Install a 12 x 75 mm tube containing 3 mL of 10% bleach on the Sample Injection Port (SIP).
- 5.4.2 Leave the support arm to the side for 1 minute.
- 5.4.3 Place the support arm under the tube, make sure the cytometer is in **RUN mode**, and let it run at **HI** flow rate for 5 minutes.
- 5.4.4 Repeat steps 5.4.1 through 5.4.3 using distilled water.
- 5.4.5 Leave a tube containing 1 mL of distilled water on the SIP with the support arm under the tube.
- 5.4.6 Set the cytometer to **STANDBY** mode.
- 5.4.7 Empty the waste tank.

5.4.8 Turn **OFF** the cytometer.

6.0 Maintenance

6.1 FACSCalibur Monthly Maintenance

- 6.1.1 Remove the sheath reservoir.
- 6.1.2 Bypass the sheath filter to avoid damage.
- 6.1.3 Install a reservoir containing 10% bleach.
- 6.1.4 Install a 12 x 75 mm tube containing 3 mL of 10% bleach on the Sample Injection Port (SIP).
- 6.1.5 Run for 20 to 30 minutes at **HI** flow rate.
- 6.1.6 Remove the tube of bleach and the bleach reservoir.
- 6.1.7 Repeat steps 6.1.3 through 6.1.3 using distilled water instead of bleach.
- 6.1.8 Place the original sheath reservoir and reconnect the sheath filter.
- 6.1.9 Perform instrument startup procedure before running samples.

6.2 Periodic calibrations (with a one-year duration) and any repairs are performed by a qualified Becton Dickinson technician upon BDP's request.

7.0 Documentation

7.1 Record use, preventative maintenance, and standardization of the instrument in the equipment Logbook as per **SOP 21531 - Equipment Logs**.

7.2 Generate and maintain all documentation relevant to this SOP according to **SOP 21409 - Good Documentation Practices**.

8.0 Reference

8.1 BD Biosciences FACS Training Manual