1 Starting Up

1. Make sure that the software protocol disk for BD FACSCount is in the drive.
2. Turn on the power to the instrument.
3. Fill the system fluid reservoir with BD FACSFlow™ sheath fluid.
4. Empty the waste reservoir.
   CAUTION: Do not dispose of waste reservoir contents until at least 30 minutes after the completion of the last run. This helps inactivate biohazardous materials before disposal.
5. Add 200 mL of BD FACSCount solution or undiluted bleach to the empty reservoir.
6. Check for air in the flow cell and prime the system if necessary.

2 Preparing Controls and Samples

Collect blood in BD Vacutainers® EDTA tubes or equivalent. Prepare controls and patient samples by adding blood, then fixative solution to the reagent tubes. Before running controls, add control beads.

Preparing Tubes

1. For the controls, label the two reagent pairs tabs.
   - CD4-Zero, CD8-Low
   - CD4-Medium, CD8-High

2. For each patient specimen, label a reagent pair tab with the patient ID or accession number.

Adding Blood to Controls

1. Invert the normal whole blood BD Vacutainer EDTA tube 5 to 10 times to adequately mix.
2. Reverse pipette 50 µL of specimen whole blood into each reagent tube labeled with the corresponding identification number. Change tips between tubes.
3. Cap the tubes and vortex the pairs upright for 5 seconds.
4. Repeat steps 1 through 3 to prepare sample tubes for each patient specimen. Change tips between tubes.

Adding Control Beads

1. Place the Zero/Low and Medium/High control beads in the control area of the workstation. See the user's guide if you are opening control beads for the first time.
2. Uncap the tubes and reverse pipette 50 µL of fixative solution into each tube. Change tips between tubes.
3. Recap the tubes and vortex the pairs upright for 5 seconds.
4. Repeat steps 1 through 3 to prepare sample tubes for each patient specimen. Change tips between tubes.

Adding Blood to Samples

1. Invert the patient specimen BD Vacutainer EDTA tube 5 to 10 times to adequately mix.
2. Reverse pipette 50 µL of specimen whole blood into each reagent tube labeled with the corresponding identification number. Change tips between tubes.
3. Cap the tubes and vortex the pairs upright for 5 seconds.
4. Repeat steps 1 through 3 to prepare sample tubes for each patient specimen. Change tips between tubes.

Adding Fixative

1. Uncap the tubes and reverse pipette 50 µL of fixative solution into each tube. Change tips between tubes.
2. Recap the tubes and vortex the pairs upright for 5 seconds.

Adding Control Beads

1. Place the Zero/Low and Medium/High control beads in the control area of the workstation. See the user's guide if you are opening control beads for the first time.
2. Uncap the tubes labeled Zero, Low, Medium, and High.
3. Vortex the Zero/Low control bead pair for 5 seconds and reverse pipette 50 µL of Zero control beads into the tube labeled Zero. Change tips between tubes.
4. Reverse pipette 50 µL of Low control beads into the tube labeled Low. Change tips between tubes.
5. Vortex the Medium/High control bead pair for 5 seconds and reverse pipette 50 µL of the Medium control beads into the tube labeled Medium. Change tips between tubes.
6. Reverse pipette 50 µL of the High control beads into the tube labeled High.
7. Recap the tubes.

6 Maintenance

Long Cleaning

Perform the following steps to clean the fluidics once a month or every 300 samples, whichever occurs first. Additionally, perform the long cleaning procedure when instructed to do so by your BD service representative.
1. Remove the sheath tank and discard the solution.
2. Rinse the tank, and fill it with 2 liters of BD FACSCount solution or a 1:10 bleach solution.
3. Place the sheath tank back on the instrument, bypassing the sheath filter.

CAUTION: Bypass the saline filter to prevent damage from cleaning and rinsing solutions.

4. Empty the waste tank and reconnect.
5. Perform the instrument daily cleaning and shutdown procedure with only the rinsing solution for five times for a total of 30 minutes (see Daily Cleaning above).
6. Replace the cleaning solution in the sheath tank with BD FACSCount solution or distilled water.
7. Perform the daily cleaning procedure with only the rinsing solution for five times for a total of 30 minutes.
8. Empty the sheath tank, and fill it with BD FACSFlow sheath fluid.
9. Connect the sheath tank and reconnect the saline filter connector to its original location.
10. Perform the shutdown procedure (see Daily Cleaning above).

CAUTION: Do not dispose of waste reservoir contents until at least 30 minutes after the completion of the last run. This helps inactivate biohazardous materials before disposal.

NOTE: Stained controls can be stored up to 24 hours before adding to the BD FACSCount™ instrument.

NOTE: Run controls on the BD FACSCount™ instrument within 2 hours of preparation.

Incubating Tubes

Incubate controls and samples in the dark for 60 to 120 minutes at room temperature.

4 Running Samples

1. Press [Sample] on the BD FACSCount screen.
2. Enter or verify the reagent lot code and reference bead counts and press [Confirm].
3. Enter the patient ID or accession number on the Sample screen.
4. Vortex the reagent pair upright for 5 seconds.
5. Uncap the CD4 tube and place the reagent pair in the sample holder so that the CD4 tube is in the run position, and press [Run].
6. Remove the reagent pair and recap the CD4 tube.
7. Uncap the CD8 tube and place the reagent pair in the sample holder so that the CD8 tube is in the run position, and press [Run].
8. Remove the reagent pair and recap the CD8 tube.
9. Repeat steps 3 through 11 for each remaining sample pair.

NOTE: Perform instrument daily cleaning and shutdown after running controls or samples.

5 Daily Cleaning

Instrument

2. Press [Clean].
3. Place a tube of BD FACSRinse solution or distilled water in the sample holder and press [Run].
4. Place a tube of BD FACSRinse solution or distilled water in the sample holder and press [Run].
5. Place a tube of BD FACSRinse solution or distilled water in the sample holder so that the CD4 tube is in the run position, and press [Run].
6. Remove the reagent pair and recap the CD4 tube.
7. Uncap the CD8 tube and place the reagent pair in the sample holder so that the CD8 tube is in the run position, and press [Run].
8. Remove the reagent pair and recap the CD8 tube.
9. Repeat steps 3 through 8 for each remaining sample pair.

NOTE: Perform instrument daily cleaning and shutdown after running controls or samples.

Coring Station

1. Invert the coring station over a sink and allow warm water to run into the metal openings surrounding the cutters.
2. Dry the coring station with a clean, dry cloth.

Electronic Pipette

Wipe down the pipette with a soft cloth dipped in a mild detergent solution.

Shutdown

2. Press [ShutDown].
3. Place a tube of BD FACSRinse solution or distilled water in the sample holder and press [Run].
4. Leave the instrument power on to continue running samples later in the day, or turn off the instrument power to complete the shutdown.

NOTE: Perform instrument daily cleaning and shutdown after running controls or samples.

6 Maintenance

Long Cleaning

Perform the following steps to clean the fluidics once a month or every 300 samples, whichever occurs first. Additionally, perform the long cleaning procedure when instructed to do so by your BD service representative.
1. Remove the sheath tank and discard the solution.
2. Rinse the tank, and fill it with 2 liters of BD FACSCount solution or a 1:10 bleach solution.
3. Place the sheath tank back on the instrument, bypassing the sheath filter.

CAUTION: Bypass the saline filter to prevent damage from cleaning and rinsing solutions.

4. Empty the waste tank and reconnect.
5. Perform the instrument daily cleaning and shutdown procedure with only the cleaning solution five times for a total of 30 minutes (see Daily Cleaning above).
6. Replace the cleaning solution in the sheath tank with BD FACSRinse solution or distilled water.
7. Perform the daily cleaning procedure with only the rinsing solution for five times for a total of 30 minutes.
8. Empty the sheath tank, and fill it with BD FACSFlow sheath fluid.
9. Connect the sheath tank and reconnect the saline filter connector to its original location.
10. Perform the shutdown procedure (see Daily Cleaning above).

Disconnect the sheath filter output tubing connector and set it aside.
• Connect the sheath tank output tubing connector to where the sheath filter output tubing connector was connected.

CAUTION: Bypass the saline filter to prevent damage from cleaning and rinsing solutions.

4. Empty the waste tank and reconnect.
5. Perform the instrument daily cleaning and shutdown procedure with only the cleaning solution five times for a total of 30 minutes (see Daily Cleaning above).
6. Replace the cleaning solution in the sheath tank with BD FACSRinse solution or distilled water.
7. Perform the daily cleaning procedure with only the rinsing solution for five times for a total of 30 minutes.
8. Empty the sheath tank, and fill it with BD FACSFlow sheath fluid.
9. Connect the sheath tank and reconnect the saline filter connector to its original location.
10. Perform the shutdown procedure (see Daily Cleaning above).

CAUTION: Bypass the saline filter to prevent damage from cleaning and rinsing solutions.

4. Empty the waste tank and reconnect.
5. Perform the instrument daily cleaning and shutdown procedure with only the cleaning solution five times for a total of 30 minutes (see Daily Cleaning above).
6. Replace the cleaning solution in the sheath tank with BD FACSRinse solution or distilled water.
7. Perform the daily cleaning procedure with only the rinsing solution for five times for a total of 30 minutes.
8. Empty the sheath tank, and fill it with BD FACSFlow sheath fluid.
9. Connect the sheath tank and reconnect the saline filter connector to its original location.
10. Perform the shutdown procedure (see Daily Cleaning above).

Disconnect the sheath filter output tubing connector and set it aside.