

BD FACSCount System Quick Reference

A Proven System for Measuring Absolute CD4, CD8, and CD3 Counts in HIV/AIDS Patients



23-8759-01

For Use with BD FACSCount™ Reagents, BD FACSCount Controls, and BD FACSCount Software
Use this after you are familiar with the procedures in the appropriate BD FACSCount system user's guide and package insert.

1 Starting Up

1. Make sure that the software protocol disk for BD FACSCount reagents 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™ FACS Clean 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 Vacutainer® 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.



3. Vortex each reagent pair upside down for 5 seconds, then upright for 5 seconds.
4. Open the reagent tubes with the coring station.



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 normal whole blood into each reagent tube labeled Zero, Low, Medium, and High. Change tips between tubes.



3. Cap the tubes and vortex the pairs upright for 5 seconds.

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 upright for 5 seconds.
4. Repeat steps 1 through 3 to prepare sample tubes for each patient specimen. Change tips between tubes.

Incubating Tubes

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

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 upright for 5 seconds.
NOTE: Stained controls can be stored up to 24 hours before adding control beads. Run samples on the BD FACSCount™ instrument within 48 hours of preparation.

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.

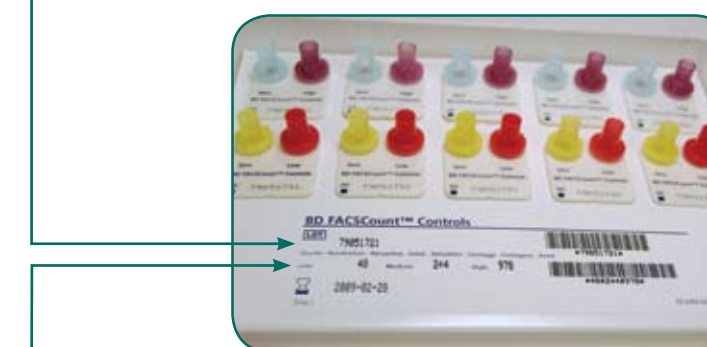


2. Uncap the tubes labeled Zero, Low, Medium, and High.
3. Vortex the Zero/Low control bead pair for 5 seconds and reverse pipette 50 µL of Zero control beads into the tube labeled Zero. Change tips between tubes.
4. Reverse pipette 50 µL of Low control beads into the tube labeled Low. Change tips between tubes.
5. Vortex the Medium/High control bead pair for five 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.

NOTE: Run controls on the BD FACSCount instrument within 2 hours of adding the control beads.

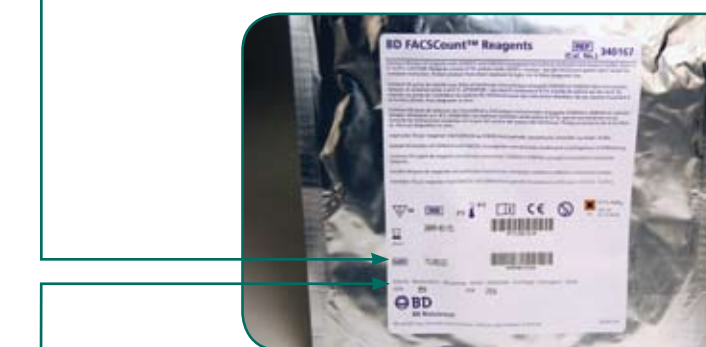
3 Running Controls

1. Press [Control] on the BD FACSCount screen.
2. Enter the eight-digit control bead lot code.



3. Enter the bead counts for the Low, Medium, and High controls and press [Confirm].

4. Enter the eight-digit reagent lot code.



5. Enter the CD4 reference bead count for the reagent lot and press [Confirm].
6. Enter the normal control ID and press [Enter].
7. Vortex the first control pair (CD4-Zero and CD8-Low) upright for 5 seconds.
8. Uncap the CD4-Zero tube and place the reagent pair in the sample holder so that the CD4-Zero tube is in the run position, and press [Run].
9. Remove the reagent pair and recap the CD4-Zero tube.
10. Uncap the CD8-Low tube and place the reagent pair in the sample holder so that the CD8-Low tube is in the run position, and press [Run].
11. Remove the reagent pair and recap the CD8-Low tube.
12. Follow steps 7 through 11 for the second pair of controls (CD4-Medium and CD8-High).

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

5 Daily Cleaning

Instrument

1. Press [Utility] on the BD FACSCount screen.
2. Press [Clean].
3. Place a tube of BD FACS Clean solution or a 1:3 dilution of bleach in the sample holder and press [Run].
4. Place a tube of BD FACS Rinse solution or distilled water in the sample holder and press [Run].

Workstation

Wipe down the workstation with a soft cloth dipped in a 1:10 dilution of bleach.

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

NOTICE: Perform daily cleaning before you turn the instrument off at the end of each day.

1. Press [Utility] on the BD FACSCount screen.
2. Press [Shutdown].
3. Place a tube of BD FACS Rinse 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.

6 Maintenance

Long Cleaning

Perform the following steps to clean the fluidics once a month or every 500 samples, whichever occurs first. Additionally, perform the long clean procedure when instructed to in Troubleshooting, or when recommended 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 FACS Clean solution or a 1:10 bleach solution.
3. Place the sheath tank back on the instrument, bypassing the sheath filter.



sheath filter bypassed

- 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.

Local BD Biosciences Office Address

Telephone Number

BD Biosciences Sales Representative

Contact Information

BD Biosciences Service Representative

Contact Information

For more information, visit bdbiosciences.com or contact your local BD Biosciences representative.