

August 2012

BD FACSCalibur™ System FAQs

The BD Biosciences online tutorials are here:

<http://www.bdbiosciences.com/services/training/elearning/index.jsp>

BD™ HLA-B27

During instrument setup, the setting of the marker is critical to correct analysis. After setup, the FL1/FITC voltages should not be altered, since this affects the marker placement and the assay.

BD Trucount™ beads (Catalog No. 340334)

What fluorescent channels do BD Trucount™ beads fluoresce in?
FITC, PE, PerCP, PE-Cy™7, APC, and APC-H7. The beads do not exhibit fluorescence in the violet laser.

BD FACSCount™ Electronic Pipettor

Has the catalog number for the BD FACSCount pipettor changed?
The new catalog number for the pipettor is 646539. The old number was 343246.

What tips can I use with the BD FACSCount pipettor?
Always use Biohit brand tips with this pipettor. Other brand tips will cause incorrect volume dispensing and inaccurate results.

It is not working properly, can I get it repaired?
If your product needs service and is not covered by a BD contract, contact Sartorius Biohit, <http://www.biohit.com/us>, for a repair quote. Customers can also contact BD Instrument Support for a refurbished pipettor.

Testing Pipetting Precision

How can we test the precision of our pipets?
The precision of pipetting should be evaluated periodically (eg, recommended monthly) to ensure the accuracy of results. Retain all records of this evaluation procedure for quality assurance purposes.

1. Select a volume normally used in the performance of the assay.
2. Using the reverse pipetting technique, pipette 10 replicates of blood and record the weights.
3. (Optional) For methods in which the beads must be pipetted into the tubes, using the reverse pipetting technique, pipette 10 replicates of bead suspension and record the weights.
4. Calculate the mean, standard deviation, and coefficient of variation (%CV). The %CV for replicates should be <2%.



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Testing Pipetting Accuracy

The following procedure can be used to test the pipet and how accurately it measures volume. Water is used because the weight of 1 μL of water is 1 μg . You must use a 4-place decimal digital scale. This is required to achieve the accuracy required to calculate %CV.

1. Using the reverse pipetting technique, pipette 10 replicates of distilled water and record the weight (100 μL of water should weigh 0.1000 grams).
2. Calculate the mean, standard deviation, and %CV. The %CV must be <2%.

Pipetting Guidelines

The following information is consolidated from operational instruction manuals from several pipet manufacturers. Complete information and more detailed instructions are contained in specific pipet instruction manuals; some of these are available online. Read the manufacturer's manual carefully before beginning the pipetting procedure.

- Select the volume you want (with manual pipets, higher volumes should be set first; if adjusting from a lower to a higher volume, first surpass the final volume and then slowly decrease the volume until the required setting is reached).
- If applicable, select the mode (eg, reverse pipetting). This is recommended for optimal precision and reproducibility.
- Reverse pipetting can be done with a manual pipet by pressing the control button to the second stop when aspirating, taking up more liquid than will be dispensed, and then pressing the control button only to the first stop when dispensing. A small volume will remain in the tip after dispensing.
- Select an appropriate tip (usually the tip color matches the color of the control button).

Pre-rinsing Guidelines

The following guidelines will help ensure optimal precision and accuracy.

- Volumes >10 μL : Pre-rinse the pipet 2–3 times for each new tip (this involves aspirating and dispensing liquid several times). Reasons for pre-rinsing include the following:
 - To compensate for system pressure, for slight differences in temperature between the pipet and the liquid, and for properties of the liquid;
 - To clear the thin film formed by the liquid on the inside of the pipet. Without pre-rinsing, retention of a thin film on the inside wall of the tip would cause the first volume to be too small. The thickness and nature of this film, and therefore the potential source of error, varies depending on the nature of the liquid being pipetted.
- Volumes <10 μL : Do not pre-rinse the pipet, but rinse the tip after dispensing to ensure that the entire volume was dispensed. For smaller volumes, pre-rinsing is not recommended because the dispensed volume would be too great.

Filling Guidelines

- Make sure the tip is securely attached.
- Hold the pipet upright.
- When aspirating, try to keep the tip at a constant depth below the surface of the liquid.
- Glide the control button slowly and smoothly (electronic pipets perform this step automatically).



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- When pipetting viscous liquids (eg, whole blood), leave the tip in the liquid for 1–2 seconds after aspirating before withdrawing it.
- After liquid is in the tip, never place the pipette on its side.

Dispensing Guidelines

- Hold the tip at an angle, against the inside wall of the vessel or tube if possible.
- Glide the control button slowly and smoothly (electronic pipettes perform this step automatically).

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