BD Leucocount™ Application Guide

For BD FACSLyric™ Flow Cytometers

23-24010(04)
2023-08
English

CE IVD Rx Only

Regulatory information

For In Vitro Diagnostic Use.

Laser safety information

The BD FACSLyric™ flow cytometer is a Class 1 Laser Product.

History

Revision	Date	Change made
23-24010(03)	2022-08	Initial release
23-24010(04)	2023-08	Updated legal manufacturer address. Added EU and Swiss importer addresses.

Contents

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Introduction

This chapter covers the following topics:

About this guide

What's in this guide

This guide covers the acquisition and analysis workflows for the BD Leucocount™ Assay using BD FACSuite™ Clinical application and describes the laboratory report. It also includes assay-specific troubleshooting information.

Assumptions

This guide assumes that you have read the *BD FACSLyric™ System Instructions For Use* (IFU) and the *BD FACSLyric™ Clinical Reference System* and that you are familiar with running the software and cytometer. The documents provide details on performing quality control (QC), creating the worklist, and running samples.

Additional information

See the *BD Leucocount™ Kit* IFU for information on preparing samples.

Technical support

Before contacting technical support

Try the following options for answering technical questions and solving problems:

- Read the section of this guide specific to the operation you are performing.
- See the assay-specific troubleshooting section of this guide for specific problems.
- See the troubleshooting section of the *BD FACSLyric™ System Instructions For Use* and the *BD FACSLyric™ Clinical Reference System.*

Contacting technical support

To contact technical support:

1. Go to bd.biosciences.com/en-us.
2. Select your region, as needed.
3. Click **Support.**
4. Click **Contact Us** on the left side of the page.
5. If needed, expand your country for details for your local region.

When contacting BD Biosciences, have the following information available:

- The product name, part number, serial number, and details of recent system performance
- The test you are performing
- Any error messages

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BD Leucocount™ Assay

This chapter covers the following topics:

About the assay

The BD Leucocount™ Kit is used to count residual white blood cells (rWBCs) in leucoreduced blood products.

Overview of the test

The BD Leucocount™ Reagent contains the nucleic acid dye, propidium iodide (PI), and RNAse. When used with RNAse, PI stains only cellular DNA. White blood cells are nucleated cells that contain DNA and therefore stain with the dye. Non-nucleated particles, such as platelets and red blood cells (RBCs), do not stain with PI.

BD Trucount™ Tubes contain a lyophilized pellet of fluorescent beads. During incubation of the reagent and the specimen, the bead pellet dissolves, releasing a known number of fluorescent beads, which are distinguished from cells by their fluorescence intensity. The stained sample is acquired on a flow cytometer and the software determines the rWBC count by comparing cellular events to bead events, and reports the counts in the lab report.

Note: Dispose the contents of the waste container in accordance with any applicable regulations. Do not discharge the liquid waste down the drain where prohibited.

Assay workflow

The following table lists the steps in a typical assay workflow.

Step	Description
1	Enter reagent lot and expiration date in the Library. See Adding reagent information to the library (page 8).
2	Perform daily instrument Performance QC (PQC) using BD® CS&T Beads. See the *BD FACSLyric™ System Instructions For Use.*
3	Perform Assay/Tube Settings Setup for Leucocount Tube Settings using BD® CS&T Beads. See the *BD FACSLyric™ Reference System*. A QC message will be generated in BD FACSuite™ Clinical application v1.4 and v1.5. See QC messages: General warnings (page 18).
4	Prepare the process controls and test specimens. See the *BD Leucocount™ Kit* IFU for information.
5	Create worklist for the process controls and test specimens. See Running the assay (page 9) or the *BD FACSLyric™ System Instructions For Use.*
6	Acquire samples. See Running the assay (page 9) or the *BD FACSLyric™ System Instructions For Use.*
7	Review the laboratory report. See Reviewing the lab report (page 12).
8	Adjust gates, if necessary. See Adjusting gates (page 13).

Adding reagent information to the library

Before you begin, you must add the lot and expiration date for the BD Leucocount™ Reagent and BD Trucount™ Tubes to the Library.

To add a reagent lot ID and expiration date to the library:

1. From the BD FACSuite™ Clinical application navigation bar, click the **Library** icon.

   The Library workspace opens.

2. Expand the **Beads and Reagents** menu and select **Reagents**.

3. Select Leucocount from the **Product Name** list.

   The reagent pane opens at the bottom of the page.

4. Click **Add Lot**.

   The **Add New Lot** dialog opens.

5. In BD FACSuite™ Clinical application v1.5, or later, click **Scan Barcode** and then scan the barcode on the vial label.

   The Lot ID and expiration date are entered in the appropriate fields.

   Note: In BD FACSuite™ Clinical application v1.4, add the Lot ID and expiration date manually.

6. Select the **Current Lot** checkbox.

7. Click **OK**.

   The lot ID and expiration date are added to the appropriate columns for the reagent.

   Note: Make sure to add the reagent lot and expiration date for BD Leucocount™ Reagent prior to acquisition. This has to be done only once for a particular reagent lot.

To add BD Trucount™ Tubes information to the Library:

1. From the BD FACSuite™ Clinical application navigation bar, click the **Library** icon.

   The Library workspace opens.

2. Expand the **Beads and Reagents** menu and select **Trucount Tubes.**

   The Trucount Tube Lot pane opens at the bottom of the page.

3. Click **Add**.

4. Enter the relevant information in the following fields: **Lot ID**, **Expiration Date**, and **Beads / Pellet**.

   The information is found on the BD Trucount™ Tubes pouch label.

5. Ensure the **Current Lot** checkbox is checked.

6. Click **Done**.

Running the assay

To run the BD Leucocount™ Assay, you create a worklist and then acquire the samples.

Before you begin

- Ensure that the current lot and expiration date for BD Leucocount™ Reagent and BD Trucount™ Tubes are entered in the Library. See Adding reagent information to the library (page 8).
- Verify that Characterization QC (CQC) has not expired. Perform CQC, if needed.
- Verify that the reference settings have not expired. Create or update reference settings using BD® FC Beads, if needed.

  Note: Reference settings are not required to run the BD Leucocount™ Assay, however, in BD FACSuite™ Clinical application v1.4 and v1.5, a QC message is generated. If you want to avoid getting the QC message, you can:

  - Update the reference settings using BD® FC Beads.
  - Add propidium iodide to the reference settings. See the *BD FACSLyric™ System Instructions for Use*.
- Perform daily Performance QC (PQC) using BD® CS&T Beads.
- Perform Assay/Tube Settings Setup for Leucocount Tube Settings. We recommend selecting the Run Setup and Generate Reports checkboxes. A QC message will be generated in BD FACSuite™ Clinical application v1.4 and v1.5. See QC messages: General warnings (page 18).
- If needed, set applied gate positions for the assay using BD Leucocount™ RBC Control or BD Leucocount™ PLT Control.

See the *BD FACSLyric™ System Instructions for Use* and the *BD FACSLyric™ Reference System* for more information.

Procedure

To create a worklist:

1. From the BD FACSuite™ Clinical application navigation bar, click the **Worklists** icon.

   The Worklists workspace opens.
2. In the **Manage Worklists** tab, click **New**.

   A blank worklist opens in a new tab.
3. In the **Worklist Entries** section, select **Leucocount** from the **Task** menu.

   Note: Create a separate task for each process control or specimen that you are running.
4. Manually enter the **Sample ID** for each task.
5. For each Leucocount sample, enter the following information, as needed:
   - Sample Name
   - Case Number
   - Keyword 1
   - Keyword 2
   - Pack Volume
6. Enter additional keywords in the provided fields, if needed.
7. Select **Manual** or **Universal Loader** for the **Loading Option**.
8. If using the Loader, select **30 Tube Rack** or **40 Tube Rack** for the **Carrier Type**.

   See the *BD FACSLyric™ System Instructions For Use* for more information.
9. (Optional) Name the worklist as needed.

To acquire the samples:

1. Set the run pointer to the sample you want to run.

   Note: Acquire the PLT or RBC Control samples and confirm that they pass before acquiring Leucocount specimens.
2. In the **Worklist Controls** bar, select **Run Selected** from the **Run** menu.

See the *BD FACSLyric™ System Instructions for Use*.

3. Vortex each stained tube 3–5 seconds at low speed immediately prior to acquisition.

   If using the BD FACS™ Universal Loader, vortex tubes immediately before placing them in the Loader racks.

4. Review the lab report and confirm that the values are within the ranges shown on the Residual WBC Assay Values and Expected Ranges sheet, provided with the process controls.

5. Run the specimens on the worklist.

   Note: If using the BD FACS™ Universal Loader, make sure that all of the tubes in the rack are acquired within the recommended age of stain. If not, you must validate tubes acquired outside the recommended time.

6. If acquiring tubes manually, follow the prompts in the software to load or unload tubes.

   Confirm that the LED status indicator, located at the base of the manual tube port, is green before you load a tube. See the *BD FACSLyric™ System Instructions For Use* for more information.

   Acquisition will continue until the stopping criterion is met:

   - Trucount beads: 10,000 events collected

   If the stopping criterion is not met, acquisition will stop after 5 minutes. See QC messages: Beads gate (page 17).

7. Examine each dot plot

   See the *BD FACSLyric™ System Instructions For Use* for more information.

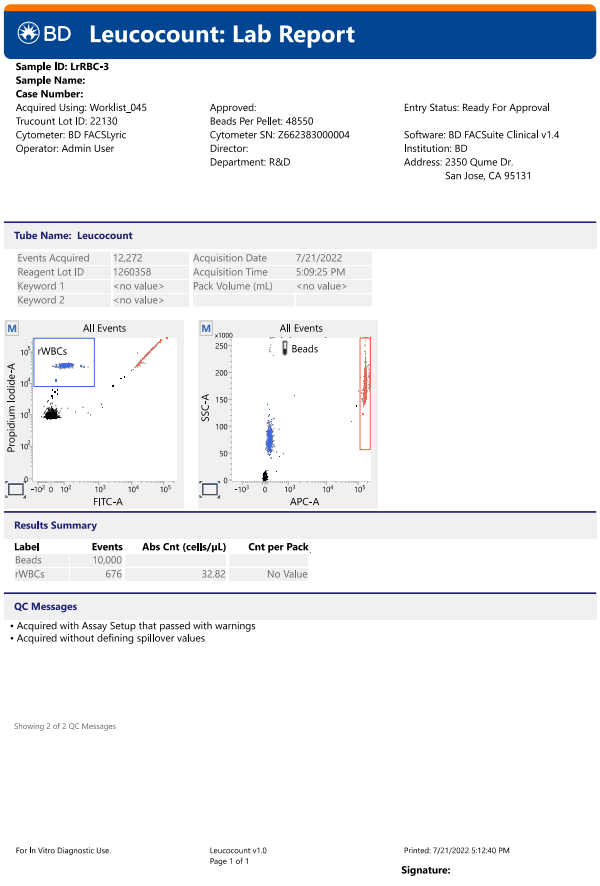
Reviewing the lab report

The BD Leucocount™ Lab Report contains assay and patient-specific information, reagent lot information, a results summary showing cell population statistics, QC messages, and dot plots with gates used to analyze the sample.

Viewing the lab report

To view the lab report:

1. Click the **Lab Report** tab to open the report.
2. Review the lab report.
   a. Review the information about the cytometer, reagents, and sample for accuracy.
   b. Inspect the dot plots and adjust the gates if needed. See Adjusting gates (page 13).
   c. Review the Results Summary showing the rWBC absolute count and the total rWBC count in the pack.
   d. Review any QC messages to address potential issues and determine whether they affect the results. See Troubleshooting (page 15)


BD Leucocount: Lab Report

Sample ID: LrRBC-3
Sample Name:
Case Number:
Acquired Using: Worklist_045
Trucount Lot ID: 22130
Cytometer: BD FACSLyric
Operator: Admin User

Approved:
Beads Per Pellet: 48550
Cytometer SN: Z662383000004
Director:
Department: R&D

Entry Status: Ready For Approval

Software: BD FACSuite Clinical v1.4
Institution: BD
Address: 2350 Qume Dr.
San Jose, CA 95131

Tube Name: Leucocount

Events Acquired	12,272	Acquisition Date	7/21/2022
Reagent Lot ID	1260358	Acquisition Time	5:09:25 PM
Keyword 1	<no value>	Pack Volume (mL)	<no value>
Keyword 2	<no value>		

Results Summary

Label	Events	Abs Cnt (cells/µL)	Cnt per Pack
Beads	10,000		
rWBCs	676	32.82	No Value

QC Messages

- Acquired with Assay Setup that passed with warnings
- Acquired without defining spillover values

Showing 2 of 2 QC Messages

For In Vitro Diagnostic Use.
Leucocount v1.0
Page 1 of 1
Printed: 7/21/2022 5:12:40 PM
Signature:

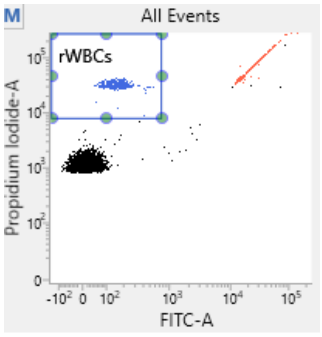
3. Approve the report.

Adjusting gates

The provided gates are automatically set, however, they might have to be adjusted for some sample types. Verify that populations on all dot plots are properly gated before reporting the results.

To resize or move a gate:

1. Click on a gate in the dot plot so that the gate is in bound mode.

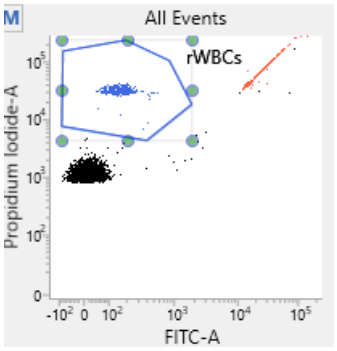


2. Click on one of the circles and drag it to resize the gate.
3. Click on one of the lines between the circles and drag it to move the gate.
4. Click inside any of the circles to rotate the gate.
5. Click the dot plot to exit bound mode.

To adjust the shape of a gate:

1. Double-click on a gate in the dot plot so that the gate is in vertex mode

   .



2. Click on one of the circles and drag it to reshape the gate.
3. Click on one of the lines between the circles and drag it to move the gate.
4. Click inside any of the circles to rotate the gate.
5. Click the dot plot to exit vertex mode

   See the *BD FACSLyric™ Clinical Reference System* for more information.

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Troubleshooting

This chapter covers the following topics:

Troubleshooting overview

This chapter lists problems you might encounter when using the BD Leucocount™ Kit, QC messages that might be generated, and provides recommended solutions.

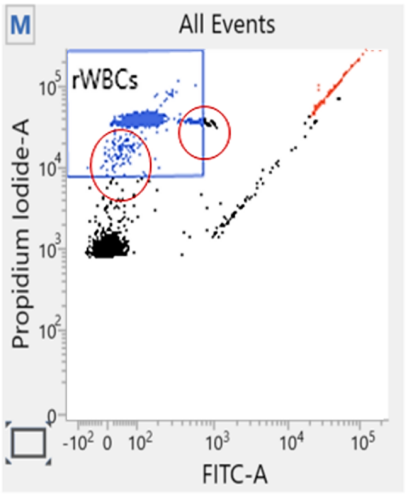
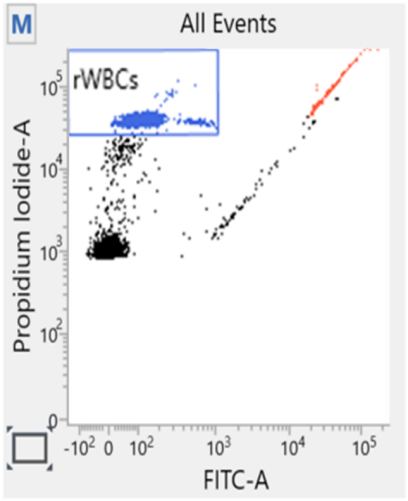
Additional troubleshooting information

The *BD FACSLyric™ Clinical Reference System* contains additional troubleshooting information covering the cytometer, setup and QC, software QC messages, and general software troubleshooting. The *BD Leucocount™ Kit* IFU also contains troubleshooting information related to the reagent and sample staining.

If after reading through the possible problems and solutions and checking the other sources of troubleshooting information you still have questions, contact BD Biosciences Technical Support. See Technical support (page 6) for information.

Troubleshooting the BD Leucocount™ Assay

Problem	Possible cause	Recommended solution
Excessive amount of debris in plots	Stained sample is too old.	See the *BD Leucocount™ Kit* IFU for information on sample stability.
	Sample was prepared improperly.	Verify the sample preparation procedure and technique.
Distorted populations or unexpected patterns in plot	Flow cell is dirty, clogged, or contains air bubbles	1. Perform a SIT flush. 2. Perform Daily Cleaning.
	Air bubbles in flow cell or in-line sheath filter.	1. Perform a SIT flush. 2. Perform Daily Cleaning.
No absolute rWBC count per pack in report	Pack volume was not entered.	Enter the pack volume in mL when filling out the worklist. See Running the assay (page 9).
No absolute rWBC count per µL in report	BD Trucount™ Tubes information was not entered in the Library.	Ensure that the BD Trucount™ Tubes information is entered in the Library before filling out the worklist.

Problem	Possible cause	Recommended solution
rWBC populations need re-gating	Specimen contains high levels of rWBCs, certain endogenous compounds, or is of poor quality.	Resize the rWBCs gate to include the positive PI population(s) and exclude streaks in the rWBCs gate. Before:  After: 

QC messages: Beads gate

QC message	Possible cause	Recommended solution
Beads Gate Failed. Place gates manually.	A BD Trucount™ Tube was not used or the bead pellet was missing from the tube.	Repeat staining using a new BD Trucount™ Tube, then acquire the newly stained sample.

QC message	Possible cause	Recommended solution
Beads gate does not contain requested 10,000 events	A BD Trucount™ Tube was not used or the bead pellet was missing from the tube.	Repeat staining using a new BD Trucount™ Tube, then acquire the newly stained sample.
Beads gate failed algorithm QC. Verify gate placement.	Unusual gate location.	Manually re-gate the sample.
	A BD Trucount™ Tube was not used or the bead pellet was missing from the tube.	Repeat staining using a new BD Trucount™ Tube, then acquire the newly stained sample.

QC messages: General warnings

QC message	Possible cause	Recommended solution
Acquired without completed Assay Setup	Assay Setup was not completed.	1. Re-run Assay/Tube Settings Setup and verify that setup is successful. 2. Re-run the sample.
Acquired with Assay Setup that passed with warnings.	BD® CS&T Beads were not prepared correctly.	1. Prepare a new tube of beads. 2. Re-run Assay/Tube Settings Setup and verify that setup is successful. 3. Re-run the sample.
Acquired with failed Assay Setup.	BD® CS&T Beads were expired.	1. Prepare a new tube of beads that are not expired and add them to the Library. 2. Re-run Assay/Tube Settings Setup and verify that setup is successful. 3. Re-run the sample.
Acquired with expired Assay Setup.	Assay Setup was not run.	1. Re-run Assay/Tube Settings Setup and verify that setup is successful. 2. Re-run the sample or stain a new specimen and acquire it.
Acquired with expired Performance QC.	Daily Performance QC (PQC) was not run.	1. Run daily PQC and verify that it passes. 2. Re-run the sample or stain a new specimen and acquire it.
Acquired with failed Performance QC.	Daily PQC failed.	1. Review QC report and address any issues with the instrument. See the *BD FACSLyric™ Clinical Reference System*. 2. Repeat PQC. Re-run the sample or stain a new specimen and acquire it.
Acquired without completed Performance QC.	Daily PQC was not completed.	1. Review QC report and address any issues with the instrument. See the *BD FACSLyric™ Clinical Reference System*. 2. Repeat PQC. Re-run the sample or stain a new specimen and acquire it.

QC message	Possible cause	Recommended solution
Acquired with Performance QC that passed with warnings.	Daily PQC passed with warnings.	1. Review QC report and address any issues with the instrument. See the *BD FACSLyric™ Clinical Reference System*. 2. Repeat PQC. Re-run the sample or stain a new specimen and acquire it.
Acquired with expired reagent: Leucocount.	The BD Leucocount™ Reagent is expired.	Re-stain sample with reagent that has not expired, and acquire the newly stained sample.
Acquired with expired Trucount bead lot.	The BD Trucount™ Tubes has expired.	Re-stain sample in a BD Trucount™ Tubes that has not expired, and acquire the newly stained sample.
Reference Settings were created with expired BD® FC Beads.	Reference settings were updated using expired BD® FC Beads.	Reference settings are not required to run the BD Leucocount™ Assay. However, to avoid getting the QC message in BD FACSuite™ Clinical application v1.4 or v1.5, you can: 1. Update reference settings using BD® FC Beads that have not expired. 2. Add propidium iodide to the reference settings. 3. Re-run the sample or stain a new specimen and acquire it.
Acquired with expired reference settings.	Reference settings have expired.	Reference settings are not required to run the BD Leucocount™ Assay. However, to avoid getting the QC message in BD FACSuite™ Clinical application v1.4 or v1.5, you can: 1. Update reference settings using BD® FC Beads. 2. Add propidium iodide to the reference settings. 3. Re-run the sample or stain a new specimen and acquire it.
Acquired without defining spillover values.	Reference settings were not created.	Reference settings are not required to run the BD Leucocount™ Assay. However, to avoid getting the QC message in BD FACSuite™ Clinical application v1.4 or v1.5, you can: 1. Create reference settings using BD® FC Beads. 2. Add propidium iodide to the reference settings. 3. Re-run the sample or stain a new specimen and acquire it.

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