BD Stem Cell Enumeration Application Guide

for BD FACSCanto II Flow Cytometers

IVD For In Vitro Diagnostic Use CE

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Regulatory information

For In Vitro Diagnostic Use.

Class 1 Laser Product.

History

Revision	Date	Change made
23-11196-00 Rev. A	4/2010	Initial release.
23-11196-01 Rev. 01	12/2012	Added Class 1 laser statement, heading for Intended Use, and Gating strategy table.

Contents

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Preface

This chapter covers the following topics:

About this guide

About this topic

This guide provides instructions for acquiring and analyzing samples stained with the BD™ Stem Cell Enumeration kit on a BD FACSCanto™ II flow cytometer using BD FACSCanto™ clinical software with BD Stem Cell Enumeration module, v1.0.

Before you begin

This guide assumes that you have read the *BD FACSCanto II Instructions for Use* and the *BD FACSCanto Clinical Software Reference Manual*, and that you are familiar with cytometer setup.

Conventions

About this topic

The following table lists the safety symbols used in this guide to alert you to potential hazards.

Safety symbols

Symbol	Meaning
	Caution alert Identifies a hazard or unsafe practice that could result in data loss, material damage, minor injury, severe injury, or death
	Biological hazard

Technical assistance

About this topic

This topic provides information on finding technical assistance.

Where to find help information

For technical questions or assistance in solving a problem:

- See Troubleshooting (page 93).
- Read the section of the *BD FACSCanto II Cytometer Reference Manual*, *BD FACSCanto Clinical Software Reference Manual*, or the *BD FACSCanto II Instructions for Use* specific to the operation you are performing.
- See the online Help in BD FACSCanto clinical software.

For more assistance

If additional assistance is required, contact your local BD Biosciences technical support representative or supplier. Visit our website, bdbiosciences.com, for up-to-date contact information.

When contacting BD Biosciences

When contacting BD Biosciences, have the following information available:

- Product name, part number, and serial number
- Any error messages
- Details of recent system performance

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Introduction

This chapter covers the following topics:

Stem Cell Enumeration overview

About this topic

This topic provides an overview of stem cell enumeration.

Overview

Transplantation of hematopoietic progenitor cells is increasingly being used in the treatment of blood disorders, malignancies, and genetic abnormalities.[1-3] The CD34 antigen is present on immature hematopoietic precursor cells and hematopoietic colony-forming cells in bone marrow and blood, including unipotent and pluripotent progenitor cells.[4] Fluorochrome-conjugated monoclonal antibodies directed against the CD34 molecule can be used to identify $CD34^+$ cells by flow cytometry.

Intended use

The BD Stem Cell Enumeration (SCE) kit provides simultaneous enumeration of viable dual-positive $CD45^+/CD34^+$ hematopoietic stem cell populations in $CD34^+$ absolute counts (cells/µL) as well as the percentage of the total viable leucocyte count that is $CD34^+$ (%CD34). The following specimens can be analyzed with this kit: normal and mobilized peripheral blood, fresh and thawed leucopheresis products, fresh and thawed bone marrow, and fresh and thawed cord blood. The kit is intended for in vitro diagnostic (IVD) use on either a BD FACSCalibur™ flow cytometer using BD CellQuest™ or BD CellQuest™ Pro software or a BD FACSCanto II flow cytometer using BD FACSCanto clinical software.

About the software

Specimens stained using the BD Stem Cell Enumeration kit and acquired on a BD FACSCanto II flow cytometer are analyzed with BD FACSCanto clinical software with BD Stem Cell Enumeration module v1.0. The software uses an automatic gating algorithm to identify and isolate stem cells. Software acquisition and analysis methods are based on the method featured in the Clinical and Laboratory Standards Institute H42-A2 approved guideline.[5]

References

No.	Citation
1	Shpall EJ, Jones RB, Bearman SI, et al. Transplantation of enriched CD34-positive autologous marrow into breast cancer patients following high-dose chemotherapy: influence of CD34-positive peripheral-blood progenitors and growth factors on engraftment. *J Clin Oncol*. 1994;12:28-36.
2	Langenmayer I, Weaver C, Buckner CD, et al. Engraftment of patients with lymphoid malignancies transplanted with autologous bone marrow, peripheral blood stem cells or both. *Bone Marrow Transplant*. 1995;15:241-246.
3	Zander AR, Lyding J, Bielack S. Transplantation with blood stem cells. *Blood Cells*. 1991;17:301-309.
4	Greaves MF, Titley I, Colman SM, et al. CD34 cluster workshop report. In: Schlossman SF, Boumsell L, Gilks W, et al, eds. *Leucocyte Typing V: White Cell Differentiation Antigens*. New York, NY: Oxford University Press; 1995;1:840-846.
5	*Enumeration of Immunologically Defined Cell Populations by Flow Cytometry; Approved Guideline-Second Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2007. CLSI document H42-A2.

Loading tubes on the cytometer

About this topic

This topic describes how to load tubes on the BD FACSCanto II flow cytometer.

Caution: Biohazard! Always wear gloves when manually loading samples on the cytometer. A fluid flush of the exterior of the sample injection tube (SIT) occurs between samples and might contain biohazardous waste.

Loading a tube

To load a tube onto the SIT:

1. When prompted, push the aspirator arm to the left.
2. Place the tube on the SIT, ensure that the tube is straight, and firmly push up until the tube comes to a complete stop and is fully seated.
3. Center the aspirator arm under the tube.

   There are three sensor pins on the aspirator arm. The bottom of the tube should sit centered above the pins.

More information

- Unloading tubes from the cytometer (page 13)
- Acquiring process controls (page 36)
- Staining and acquiring samples (page 38)

Unloading tubes from the cytometer

About this topic

This topic describes how to unload tubes from the BD FACSCanto II flow cytometer.

Caution: Biohazard! Always wear gloves when manually loading samples on the cytometer. A fluid flush of the exterior of the sample injection tube (SIT) occurs between samples and might contain biohazardous waste.

Caution! To prevent backflow into the tube, follow the tube removal sequence exactly.

Unloading a tube

To unload a tube from the SIT:

1. Wait for the aspirator arm to lower.
2. When prompted, hold the tube while pushing the aspirator arm to the left.

Caution: Biohazard If you do not hold the tube when you move the aspirator arm, the tube could fall off the SIT and expose you to potentially biohazardous sample.

3. Remove the tube from the SIT.
4. Release the aspirator arm.

   SIT cleaning occurs when the aspirator arm comes to the center.

More information

- Loading tubes on the cytometer (page 12)
- Acquiring process controls (page 36)
- Staining and acquiring samples (page 38)

Daily workflow

About this topic

This topic helps you organize your daily workflow when processing specimens for stem cell analysis and acquisition.

When running other panel types

If you will be acquiring other panel types throughout the day, optimize those settings **after** you optimize the BD Stem Cell Enumeration application. See Setting up the application (page 31). Do not re-run Standard Setup before acquiring your stem cell samples. If you do, an error message appears when you click **Run** stating that the cytometer settings for the BD Stem Cell + 7AAD panel were not optimized. The worklist will stop and will not allow you to continue until you re-run Standard Setup followed by Application Setup.

Caution! Application setup must be performed **after** Standard Setup. The software will not allow you to process samples if the BD Stem Cell.opt file is older than the SetupResults.dat file.

Workflow

The cytometer lasers must be turned on at least 30 minutes before running setup. To optimize efficiency and allow time for the lasers to warm up, we recommend performing tasks in the following order.

No.	Task	See
1	Turn on the cytometer.	Starting up the system (page 28)
2	Run Fluidics Startup.	Starting up the system (page 28)
3	Stain the optimization and process controls.	*BD Stem Cell Enumeration kit* package insert

No.	Task	See
4	Prepare the BD FACS 7-color setup beads. **Note:** Verify that the cytometer lasers have warmed up for 30 minutes before preparing the setup beads.	*BD FACS 7-Color Setup Beads kit* package insert
5	Set the following: • Print and export options • Countdown time • Ignore Loader (load tubes manually)	Setting options (page 19)
6	Enter setup beads lot information.	Entering setup bead lot information (page 21)
7	Verify that lot ID and bead/pellet values entered in the software match the values printed on the BD Trucount™ beads pouch.	Entering lot IDs (page 23)
8	Run Standard Setup and verify that it was successful.	Setting up the cytometer (page 28) Setting up the application (page 31)
9	Run Application Setup using the BD Stem Cell panel and verify that it was successful. **Note:** If you need to optimize other panels, do so after completing Application Setup, but before you exit Setup.	Setting up the application (page 31) Reviewing the Application Setup Report (page 89)
10	Set up a new Worklist for the controls, run the BD Stem Cell controls, and inspect the plots.	Acquiring process controls (page 36)

No.	Task	See
11	Verify that the control values are within the ranges shown on the BD Stem Cell Controls Assay Values sheet. **Note:** If Standard Setup is re-run after Application Setup, you will need to repeat Standard Setup and then Application Setup.	*BD Stem Cell Controls kit* package insert When running other panel types (page 14) Setting up the application (page 31)
12	Stain the patient specimens. **Caution!** Do not stain specimens until controls have been run successfully and results are within the established range for the process control lots you are using.	*BD Stem Cell Enumeration kit* package insert
13	Acquire the stained samples.	Staining and acquiring samples (page 38)
14	Review the Lab Reports.	Reviewing the Lab Report (page 91)
15	Inspect all plots and re-gate if necessary.	Re-gating plots manually (page 40)
16	Troubleshoot any problems.	Troubleshooting (page 93)
17	Once you are finished acquiring stem cell samples, clear the **Ignore Loader** option so that other operators can use the BD FACS™ Loader (Loader).	Setting options (page 19)

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Setting up the software

This chapter covers the following topics:

Workflow for setting up the software

About this topic

This topic gives an overview of the tasks involved in setting up the software. Before using the software to run stem cell samples, set options and enter default information.

Workflow

Software setup includes the following tasks.

Task	Description
1	Setting options (page 19).
2	Entering setup bead lot information (page 21).
3	Entering lot IDs (page 23).
4	Viewing acquisition targets (page 24).

Setting options

About this topic

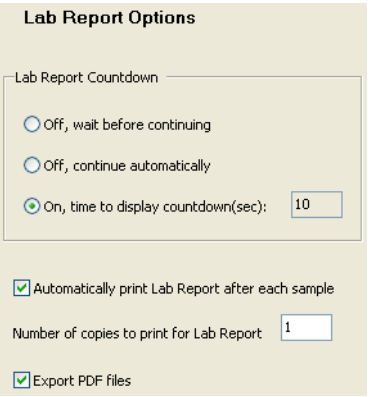
This topic describes how to set options in the software that streamline acquisition and analysis. Options include whether to:

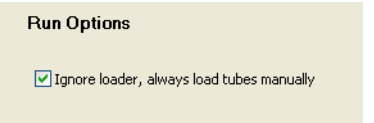
- Print Setup and Lab reports and the number of copies
- Pause the Lab Report view and for how long
- Export PDF files
- Load tubes on the cytometer manually

Procedure

To set options:

1. Select **Tools > Options**.
2. Click each icon and choose options by selecting the checkbox or button.

Option icon	Settings
 Setup	 **Note:** A printer must be attached or selected.
 Lab Report	

Option icon	Settings
 Run	 **Caution!** Due to the temperature requirements of this assay, the BD FACS Loader cannot be used to acquire samples. **Note:** After you have acquired all of your stem cell samples, clear this option so that other operators can use the Loader.

3. Click **OK** to save your selections and close the **Options** window.

More information

- Entering setup bead lot information (page 21)
- Entering lot IDs (page 23)

Entering setup bead lot information

About this topic

This topic describes how to enter bead lot information for the BD FACS™ 7-color setup beads. Bead lot information sets the detector target values and spectral overlap factors for the cytometer.

Before you begin

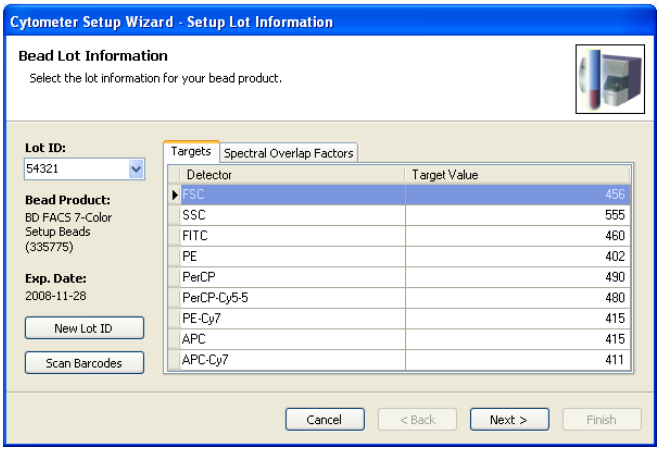
Locate the setup values card that was shipped with the new lot of BD FACS 7-color setup beads.

Procedure

To enter new bead lot information:

1. Select **Cytometer > Setup > Standard Setup**.

   The **Bead Lot Information** window appears. If the bead lot has expired, an error message appears.



2. Enter new lot information manually or by scanning barcodes. See the *BD FACSCanto II Instructions for Use* for more information.

   - Manually:

     a. Click the **New Lot ID** button, enter the lot information, and click **OK** to save the changes.

     b. Using the setup values card from the new bead lot, enter the target values in the **Targets** tab and the spectral overlap values in the **Spectral Overlap Factors** tab.

   - Scanning barcodes:

     Click the **Scan Barcodes** button, follow the prompts, and click **OK** to save the changes.

3. In the **Bead Lot Information** window, verify that the new Lot ID is selected.

4. Click **Finish**.

5. In the **Save Setup Bead Lot Info** dialog, click **Yes** to save the information you just entered.

More information

- Setting options (page 19)
- Entering lot IDs (page 23)

Entering lot IDs

About this topic

This topic describes how to enter lot IDs for BD Trucount absolute count beads and the BD Stem Cell Enumeration kit.

Before you begin

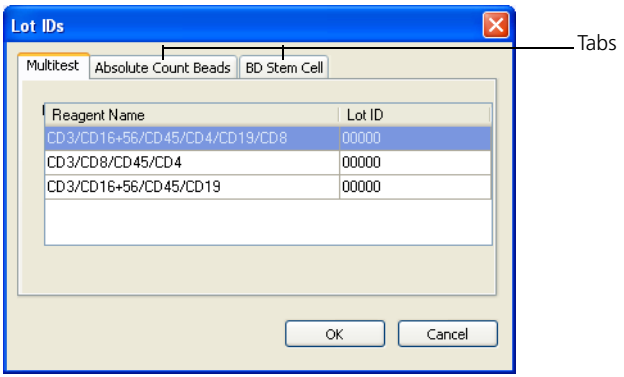
Have the BD Trucount absolute count beads pouch and the BD Stem Cell Enumeration kit box handy to enter the lot IDs.

Procedure

To enter lot IDs:

1. Select **Tools > Lot IDs**.

   The Lot IDs dialog appears.



2. Click the **BD Stem Cell** tab and enter the lot ID from the BD Stem Cell Enumeration kit.

3. Click the **Absolute Count Beads** tab, enter the lot ID from the BD Trucount beads pouch, and enter the beads per pellet value.

   Caution Verify that the values you enter match the values printed on the BD Trucount pouch. Using an incorrect bead count will result in inaccurate absolute counts.

4. Click **OK** to save the information.

More information

- Setting options (page 19)
- Entering setup bead lot information (page 21)

Viewing acquisition targets

About this topic

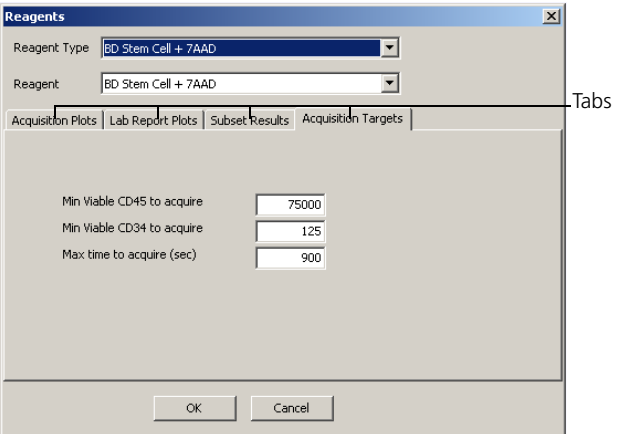
This topic describes how to view the acquisition targets set for the BD Stem Cell + 7AAD and BD Stem Cell panels. Your lab manager sets the CD45, CD34, and time targets. The BD Trucount beads target is not user-adjustable.

Procedure

To view acquisition targets:

1. Select **Reagents > BD Stem Cell + 7AAD** (or **BD Stem Cell**) **> Acquisition Targets**.

   The **Reagents** dialog appears.



The default acquisition targets are set using the following criteria:

- Minimum viable CD45 is set to 75,000 based on the recommendation in the Clinical and Laboratory Standards Institute H42-A2 approved guideline.[1]
- Minimum viable CD34 is set to 125 to ensure that at least 100 CD34 cells are acquired after final analysis.
- Maximum time is set to 900 seconds to ensure that acquisition target values are met without depleting sample.
- BD Trucount beads count is set to 1,000 to ensure that sufficient beads are acquired to accurately calculate absolute counts.

2. Click **OK** to close the dialog.

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1. *Enumeration of Immunologically Defined Cell Populations by Flow Cytometry; Approved Guideline-Second Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2007. CLSI document H42-A2.

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Cytometer setup

This chapter covers the following topics:

Starting up the system

About this topic

This topic describes how to start up the computer, software, and cytometer. Be sure to allow the cytometer lasers to warm up for 30 minutes before running setup.

Procedure

To start up the system:

1. Turn on the power to the computer and the BD FACSCanto II flow cytometer.
2. Start BD FACSCanto clinical software and log in.
3. Run fluidics startup.
4. Allow the cytometer to warm up for 30 minutes before running setup.

More information

- Workflow for setting up the software (page 18)
- Setting up the cytometer (page 28)

Setting up the cytometer

About this topic

This topic describes how to set up the cytometer.

Before you begin

- Prepare BD FACS 7-color setup beads according to the instructions in the reagent package insert.
- Read the instructions in Loading tubes on the cytometer (page 12) and Unloading tubes from the cytometer (page 13).

Setting up the cytometer

To set up the cytometer:

1. Verify that the cytometer is ready.
2. Select **Cytometer > Setup > Standard Setup**.

   The **Setup Lot Information** window opens.

3. Select the lot number for the BD FACS 7-color setup beads you are using from the **Lot ID** menu.

   If you need to enter new lot information, see Entering setup bead lot information (page 21).

4. Verify that the beads have not expired. (The software will indicate if the beads are expired.)

5. Verify that the values in the **Targets** and **Spectral Overlap Factors** tabs match those for the BD FACS 7-color setup beads you are using.

6. Click **Next**, verify that **Run setup in manual mode** is selected, and then click **Next** again.

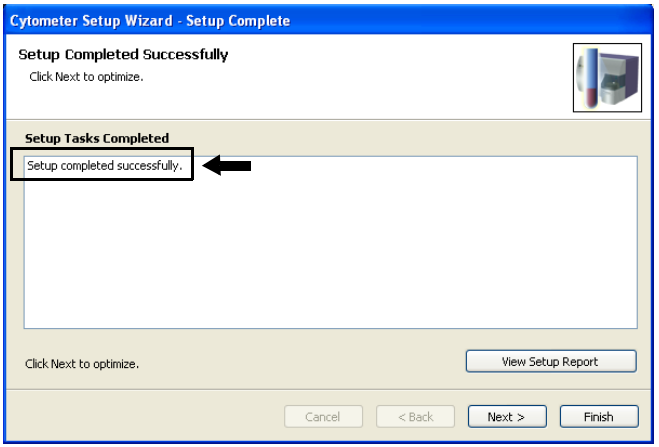
7. Load the bead tube on the cytometer when prompted and click **OK**.

   The software acquires data to set up the cytometer.

8. Remove the tube when prompted.

Verifying the setup

1. Verify that setup was successful.

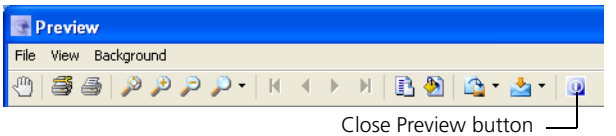


If setup was not successful, see the *BD FACSCanto II Instructions for Use* for troubleshooting information.

2. (Optional) To view the report, click **View Setup Report**.

   For a description of the elements in the report, see Reviewing the Cytometer Setup Report (page 86).

3. Click the **Close Preview** button to close the report and return to the wizard.



4. Click **Next**.
5. In the **Save Setup Results** dialog, click **Yes** to continue.
6. Click **Next** to proceed with application setup.

   The **Setup Optimization** view appears.

Next steps

Continue with Setting up the application (page 31).

More information

- Loading tubes on the cytometer (page 12)
- Unloading tubes from the cytometer (page 13)
- Setting up the application (page 31)

Setting up the application

About this topic

This topic describes how to set up the BD Stem Cell Enumeration application. Compensation values for the assay are calculated using an optimization sample.

When running other panel types

If you will be acquiring other panel types throughout the day, optimize those settings **after** you optimize the BD Stem Cell Enumeration application (see step 10 on page 33). Do not re-run Standard Setup before acquiring your stem cell samples. If you do, an error message appears when you click **Run** stating that the cytometer settings for the BD Stem Cell + 7AAD panel were not optimized. The worklist will stop and will not allow you to continue until you re-run Standard Setup followed by Application Setup.

Caution! Application Setup must be performed **after** Standard Setup. The software will not allow you to process samples if the BD Stem Cell.opt file is older than the SetupResults.dat file.

Before you begin

- Stain either the High or the Low BD Stem Cell control with BD Stem Cell reagent and 7-AAD to use as an optimization tube.

  Follow staining instructions and consult the Staining Summary table in the *BD Stem Cell Enumeration Kit* package insert.
- Read the instructions in Loading tubes on the cytometer (page 12) and Unloading tubes from the cytometer (page 13).
- Set up the cytometer. See Setting up the cytometer (page 28)

Procedure

To set up the application:

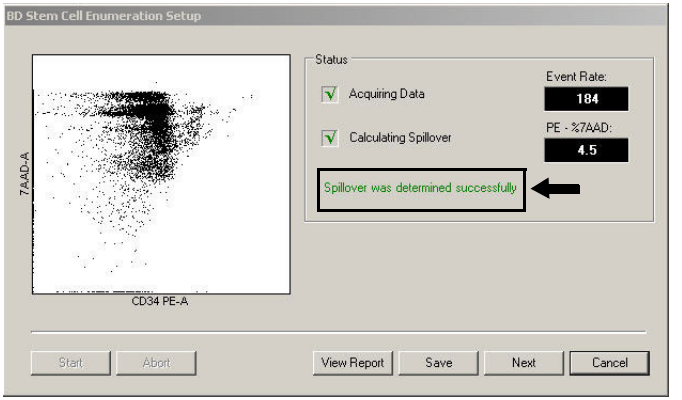
1. Select **BD Stem Cell** from the **Panel Types** menu in the Setup Optimization wizard.
2. Click **Next**.

   The **BD Stem Cell Enumeration Setup** view appears.

3. Click **Start**.
4. Load the control stained with 7-AAD (optimization) tube on the cytometer when prompted and click **OK**.

   The software begins acquiring data and then calculates spillover of 7-AAD into the PE channel.
5. Check the status message under the **Calculating Spillover** checkbox.

   - If spillover was determined successfully, go to step 6.
   - If spillover was not determined successfully, click **Cancel** and repeat step 1 through step 5. Also, see Troubleshooting (page 93).



6. Click **View Report**.

   a. Confirm that the **Overall Result** is PASS.

   b. Note the PE–%7-AAD spectral overlap value.

      A value of –0.5% to 10.5% indicates a successful setup optimization.

   For a description of the elements in the report, see Reviewing the Application Setup Report (page 89).
7. To close the report and return to the wizard, click the **Close Preview** button.

8. Click **Save**.
9. Unload the tube when prompted.
10. (Optional) Click **Next** if you have other panels to optimize.
11. Click **Finish**.

    Optimized setup results are saved to the BD Stem Cell.opt file in the C:\Program Files\Common Files\BD\Setup Results folder.

More information

- Reviewing the Application Setup Report (page 89)

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Acquisition

This chapter covers the following topics:

Acquiring process controls

About this topic

This topic describes how to acquire stained process controls on the cytometer.

Caution

Caution! Due to the temperature requirements of this assay, the Loader cannot be used to acquire samples.

Before you begin

- Stain the High and the Low BD Stem Cell controls using the BD Stem Cell Enumeration kit, but do not add 7-AAD. Follow the instructions in the reagent package insert.
- Read the instructions in Loading tubes on the cytometer (page 12) and Unloading tubes from the cytometer (page 13).
- Set up the application. See Setting up the application (page 31).

Procedure

To acquire process controls:

1. Enter the High and Low control information in the Worklist.

   Note: The combination of Name, ID, and Case Number must be unique.

   a. Enter **Control** in the **Name** column for both the High and Low controls.

      Note: If you do not enter **Control** in the **Name** column, the BD Stem Cell panel will be disabled.

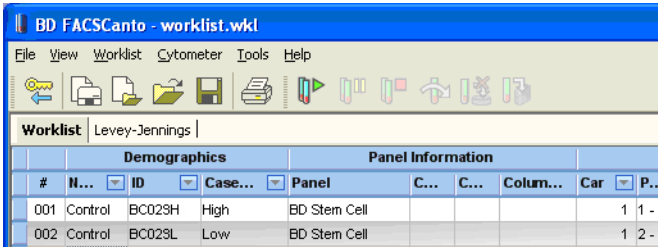
   b. Enter the lot ID number in the **ID** column.

      The Name and ID become the file name.

   c. Enter the control type (High or Low) in the **Case Number** column.

d. Select **BD Stem Cell** from the **Panel** menu.

Note: If you choose the BD Stem Cell + 7AAD panel to run the control, an error message will appear.



2. Click **Run** ( ) to acquire the High and Low controls.
3. After acquisition is complete, inspect all plots and verify that the gates enclose the appropriate populations. Adjust as necessary.

   Note: Because the process controls were run with the BD Stem Cell panel (without 7-AAD), the two viability plots (7 and 8) do not appear on the lab report.
4. Verify that the results are within the established range for the process control lots you are using.

   See the controls assay sheet for value ranges.

Next step

Continue with Staining and acquiring samples (page 38).

More information

- Loading tubes on the cytometer (page 12)
- Unloading tubes from the cytometer (page 13)
- Staining and acquiring samples (page 38)
- Re-gating plots manually (page 40)

Staining and acquiring samples

About this topic

This topic describes how to acquire stained samples on the cytometer.

Caution

Caution! Do not stain specimens until controls have been run successfully.

Caution! Due to the temperature requirements of this assay, the Loader cannot be used to acquire samples.

Before you begin

- Read the instructions in Loading tubes on the cytometer and Unloading tubes from the cytometer (page 13).
- Acquire the process controls. See Acquiring process controls (page 36).
- Review the Gating guideline (page 46) and the Overview of gating examples (page 48) for the specimen type you will be acquiring.

Procedure

To stain and acquire samples:

1. Follow the instructions in the *BD Stem Cell Enumeration kit* package insert to dilute specimens as necessary and stain them.
2. Select the **BD Stem Cell + 7AAD** panel from the **Panel** menu in the Worklist.

   Do not use the **BD Stem Cell** (without 7-AAD) panel for patient samples. This panel is to run controls only.
3. Add samples to the Worklist.

   Make sure that the combination of Name, ID, and Case number is unique for each sample.

4. If the sample required dilution, add the dilution factor to the **Dilution** column for that sample. Add specific sample information as necessary.

   For undiluted samples, use the default value of 1.

5. Click **Run** ().

6. At the prompt, choose to save changes to the Worklist.

7. Gently vortex the stained samples thoroughly, at low speed, to resuspend the cells and beads.

8. When prompted, install the tube containing the first sample in the worklist on the cytometer and click **OK**.

   The **Acquisition** tab appears and events appear in the plots.

9. Once the acquisition targets are met, the Lab Report appears.

10. Print all Lab Reports.

    See Setting options (page 19) to set print options.

11. Inspect all plots to verify that all gates include the appropriate populations. See Re-gating plots manually (page 40) if re-gating is necessary.

12. Once you are finished acquiring stem cell samples, clear the **Ignore Loader** option so that other operators can use the Loader.

    See Setting options (page 19).

More information

- Loading tubes on the cytometer (page 12)
- Unloading tubes from the cytometer (page 13)
- Acquiring process controls (page 36)
- Re-gating plots manually (page 40)

Re-gating plots manually

About this topic

You can modify gates by pausing the Worklist at the Lab Report, or you can finish running the Worklist and then modify gates during analysis.

Procedure

To modify gates by pausing the Worklist at the Lab Report:

1. Click **Pause** during the countdown between samples.
2. Click any plot to adjust its gate.

   The selected plot appears in an enlarged view.
3. Adjust gates as necessary.

   Read the instructions in the Gating guideline and examples (page 41).

   To set the gates back to the original positions, click **Autogate**.
4. Click **Continue** to close the plot.
5. Click **Run** to continue acquisition.

More information

- Staining and acquiring samples (page 38)
- Gating guideline and examples (page 41)

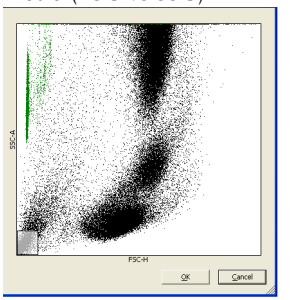
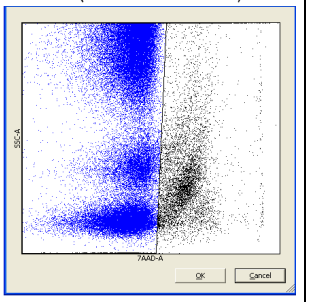
6

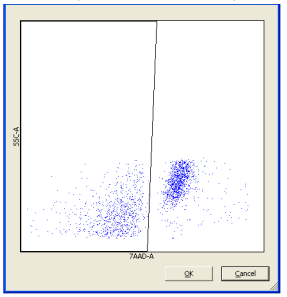
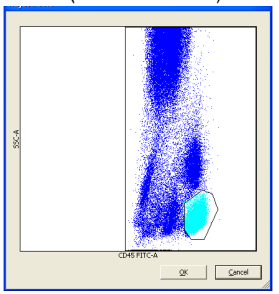
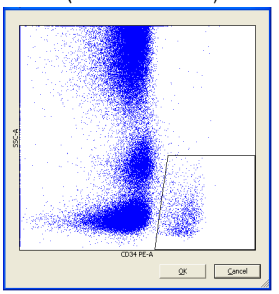
Gating guideline and examples

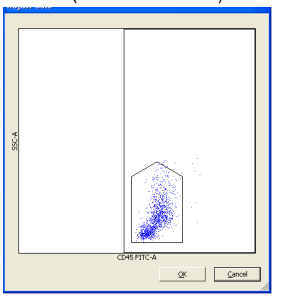
This chapter covers the following topics:

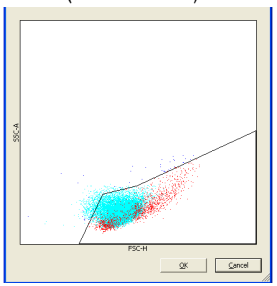
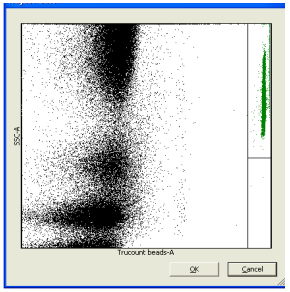
Gating strategy

Introduction This table explains the template gating strategy. See this table for general information about gating strategy. For details about specific gating examples, see Overview of gating examples (page 48).

Step	Description	Dot Plot	Plot information	Action
1.	Exclude debris from the plots displaying the cells.	Plot 6 (FSC vs SSC)  **Gate name:** Debris	• All ungated events are displayed. • Events in the Debris gate appear gray. • Beads appear green in the upper-left corner.	The Debris gate encompasses events at the lower-left corner of the plot. Adjust if necessary.
2.	Identify the viable cells in plot 8.	**Plot 8 (7-AAD vs SSC)**  **Gate name:** Viable	• All ungated events (excluding debris) are displayed. • Events within the Viable gate appear blue. **Note:** This viable gate is the same gate as shown in plot 7.	Confirm that the Viable gate encompasses only 7-AAD$^-$ events.

Step	Description	Dot Plot	Plot information	Action
3.	Identify the viable cells in plot 7.	**Plot 7 (7-AAD vs SSC)**  **Gate name:** Viable	• All CD34$^+$ events are shown, viable and non-viable. • All events are blue. **Note:** This viable gate is the same gate as shown in plot 8.	Confirm that the Viable gate encompasses only 7-AAD$^-$ events. This is the only place that non-viable CD34 events will be displayed (7-AAD$^+$).
4.	Identify the lymphocytes.	**Plot 1 (CD45 vs SSC)**  **Gate names:** CD45Pos and Lymphocytes	• All events excluding beads and debris are displayed. • CD45$^+$ events appear blue. • Lymphocytes appear light blue.	Adjust the CD45Pos gate to include all events. The left edge of the gate should include all Dim CD45 cells. Avoid platelet streaks if present.
5.	Identify the CD34$^+$ events among the viable CD45$^+$ events in plot 1.	**Plot 2 (CD34 vs SSC)**  **Gate name:** CD34Pos	• Viable CD45$^+$ events from plot 1 are displayed. • CD34$^+$ events are enclosed by the gate in the lower right of the plot.	Adjust the CD34Pos gate to include the cluster of events at the lower right of the plot. Do not reduce the height of the gate. **Note:** Non-viable CD34 events are not displayed.

Step	Description	Dot Plot	Plot information	Action
6.	Identify the $CD45^+$ dim events among the viable $CD45^+$ events in plot 1.	**Plot 3 (CD45 vs SSC)**  **Gate name:** CD45Dim	• All $CD45^+$, from the CD34 gated cells in plot 2 are displayed. • The rectangle gate is for CD45Pos events. • The polygon gate is for CD45Dim events.	Confirm that the CD45Dim gate encompasses the cluster of cells in the CD45Pos gate. **Note:** The height of the gate is the same as Plot 2. The gate is set by the algorithm on total CD34 cells which is necessary for CD34 viability estimation.

Step	Description	Dot Plot	Plot information	Action
7.	Identify stem cells as SSC^{low} among viable $CD34^{+}$ and lymphocyte events.	**Plot 4 (FSC vs SSC)**  **Gate name:** Viable CD34	• Viable lymphocytes and viable CD45Dim events are displayed. • CD34Pos events appear red.	Adjust the Viable CD34 gate to include the viable lymphocytes.
8.	Identify BD Trucount beads.	**Plot 5 (BD Trucount vs SSC)**  **Gate name:** Beads	• All ungated events are displayed. • Events in the Beads gate appear green.	Extend the Beads gate off the right edge of the plot to include all bead events.

Gating guideline

About this topic

This topic shows an example of how BD FACSCanto clinical software processes a sample stained using the BD Stem Cell Enumeration kit and analyzed on a BD FACSCanto II flow cytometer. The stained sample in this example is fresh mobilized peripheral blood.

How to use this information

Use this information to review your data plots, verify the placement and boundaries of each gate, and adjust gates as necessary.

Adjust gates **only** if necessary.

Key terms

Key terms in the gating guideline table correspond to the following information:

- **Step No.** The order in which the gating algorithm processes the data.
- **Plot No.** The order in which the plot appears in the Lab Report.
- **Plot Type.** The plot axis labels.
- **Software Action.** How the gating algorithm processes the data.
- **Gate Name.** The name of the gate as it appears on the Lab Report.
- **User Action.** What to look for on the plot and how to manually adjust the gate (if necessary).

Example Lab Report

The plots are displayed on the Lab Report in the following order.

Specimen

ALP16-BD1

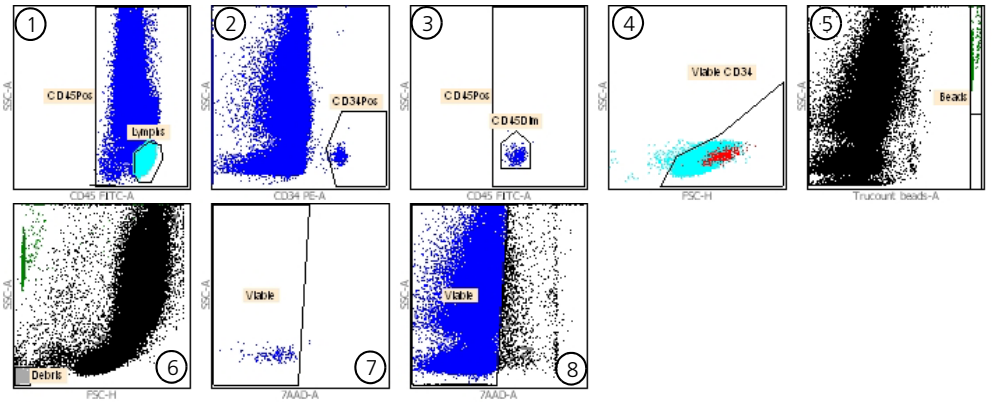
Director: BD

Panel:	BD Stem Cell + 7AAD
Acquired:	7/1/2008 10:34:08 AM
Analyzed:	7/16/2008 6:49:06 PM
TruC Lot ID:	12007
Bead/Pellet:	52000
Status:	OK
Operator:	PAL
Reviewer:	
Results:	01072008.csv

Dilution Factor: 4.0 Column #2: Column #3:

BD FACSCanto II V96322004 BD FACSCanto v.2.4.3155.23769

BD Stem Cell + 7AAD Total Events: 85211



Plot no.	Gate(s)		Plot no.	Gate(s)
1	CD45Pos, Lymphs		5	Beads
2	CD34Pos		6	Debris
3	CD45Pos, CD45Dim		7	Viable
4	Viable CD34		8	Viable

Overview of gating examples

About this topic

This topic introduces examples of gating for data acquired from different types of specimens:

Leucopheresis sample (fresh)

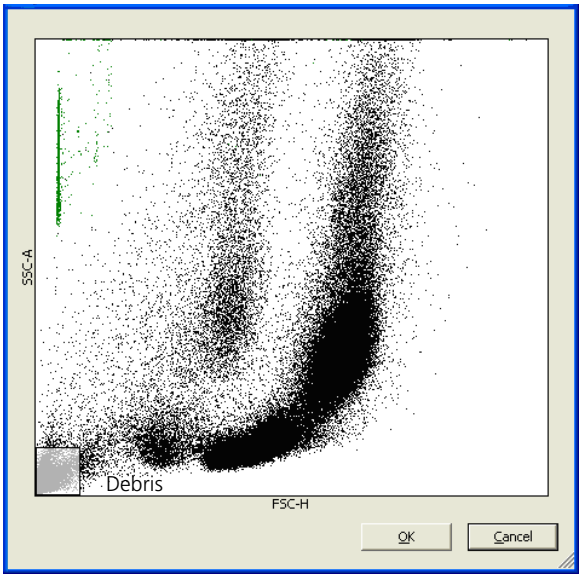
About this topic

This topic shows an example of how BD FACSCanto clinical software processes a fresh leucopheresis sample stained using the BD Stem Cell Enumeration kit and analyzed on a BD FACSCanto II flow cytometer. Gates shown are auto-gates set by the software. Manual gate adjustment was not necessary. See the Gating guideline (page 46) for more information.

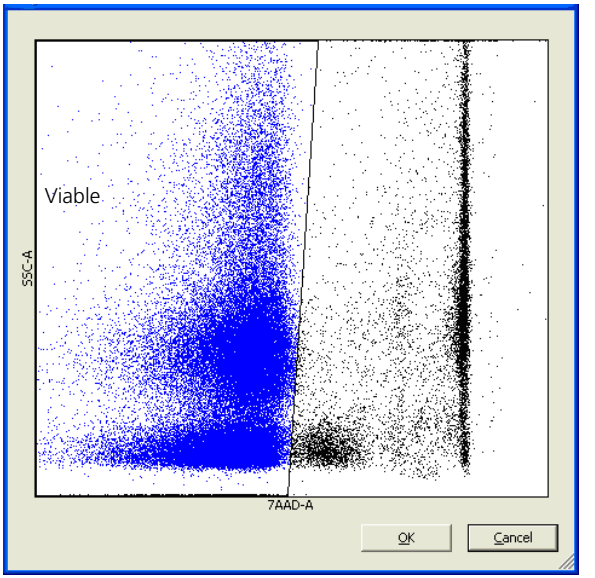
How to use this information

Use this information to review your data plots, verify the placement and boundaries of each gate, and adjust the gates as necessary.

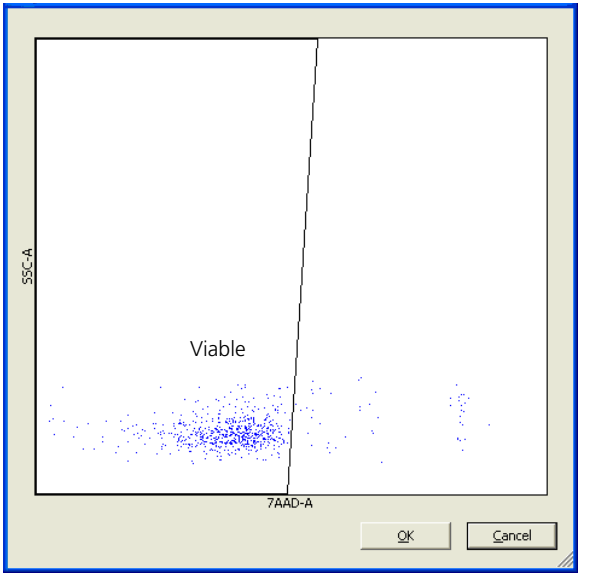
Step 1, plot 6



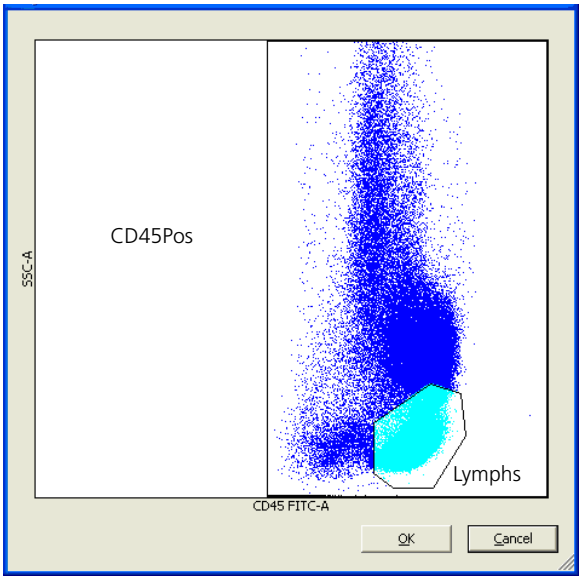
Step 2, plot 8



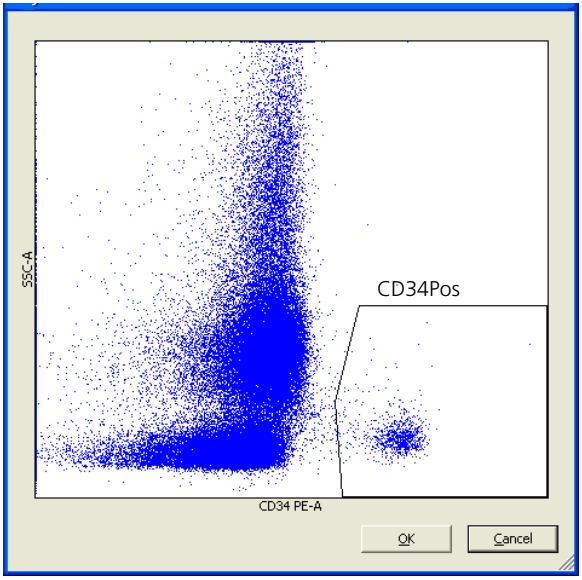
Step 3, plot 7



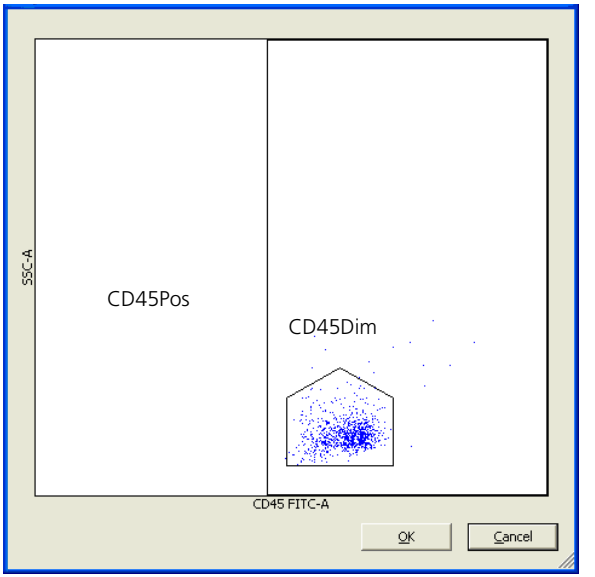
Step 4, plot 1



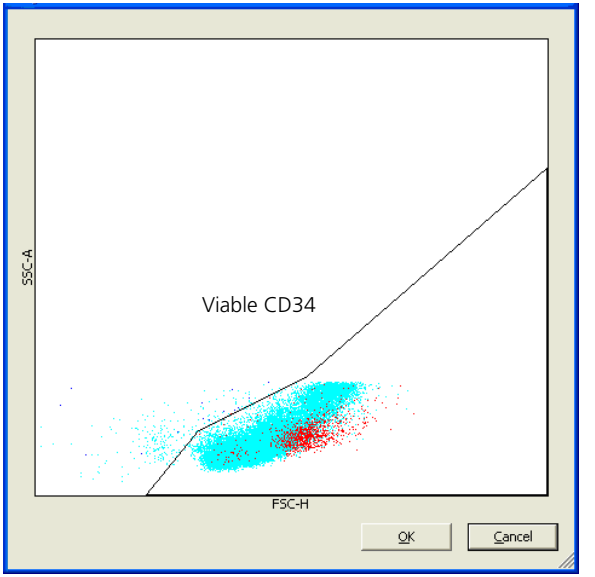
Step 5, plot 2



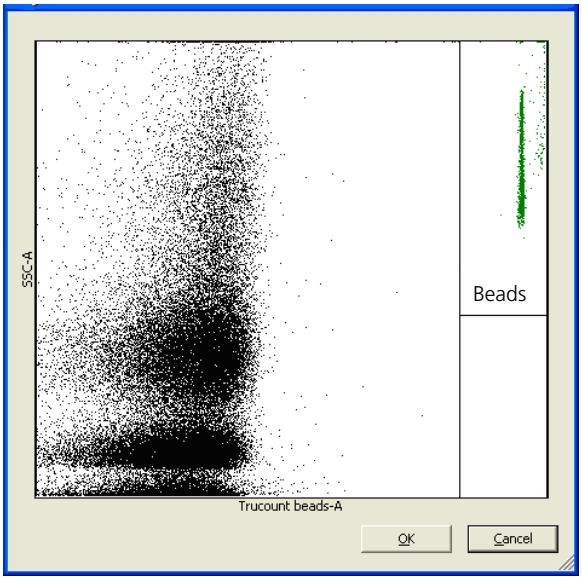
Step 6, plot 3



Step 7, plot 4



Step 8, plot 5



Leucopheresis sample (thawed)

About this topic

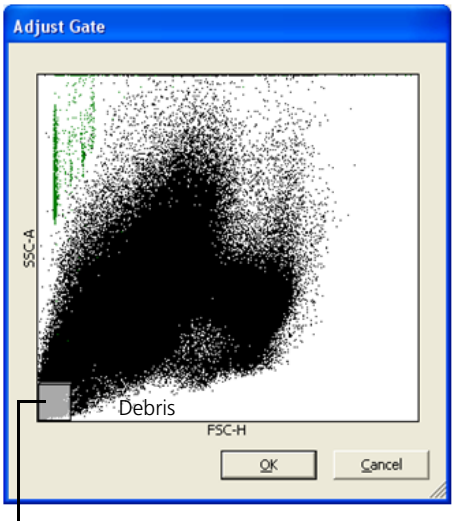
This topic shows an example of how BD FACSCanto clinical software processes a thawed leucopheresis sample stained using the BD Stem Cell Enumeration kit and analyzed on a BD FACSCanto II flow cytometer (plots are labeled Auto gating). A second set of plots (labeled Manual gating) shows an example of how to manually adjust each gate. See the Gating guideline (page 46) for more information.

How to use this information

Use this information to review your data plots, verify the placement and boundaries of each gate, and adjust the gates as necessary. See Lab Report for auto gating (page 61) and Lab Report for auto gating (page 61) to view the Lab Reports for this sample.

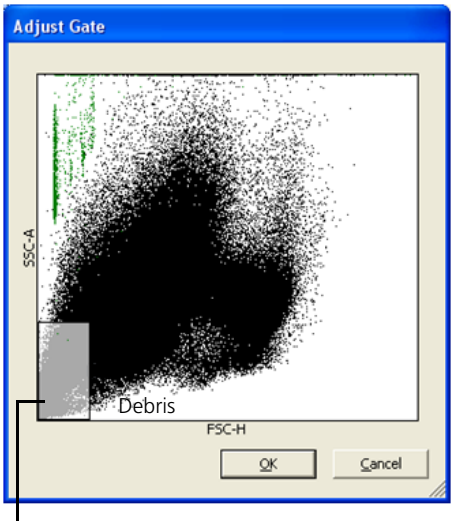
Step 1, plot 6

Auto gating



The Debris gate is too small.

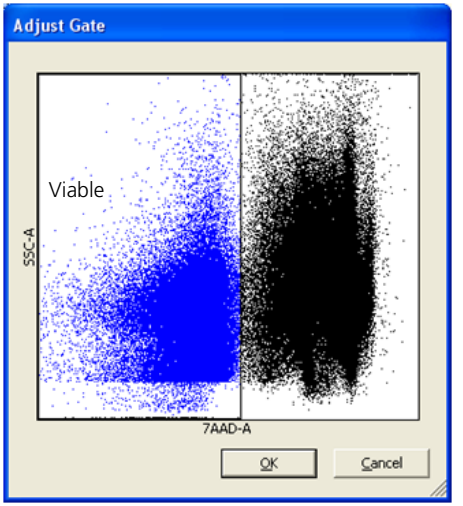
Manual gating



Increase the Debris gate. Do not include bead events. Subsequent plots will show less debris and populations of interest will be more visible.

Step 2, plot 8

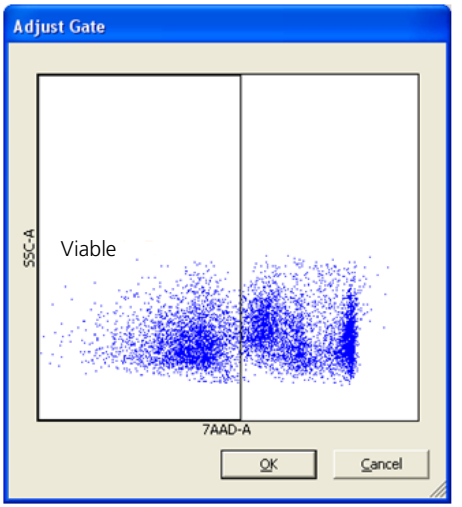
Auto gating



No manual gate adjustment is necessary.

Step 3, plot 7

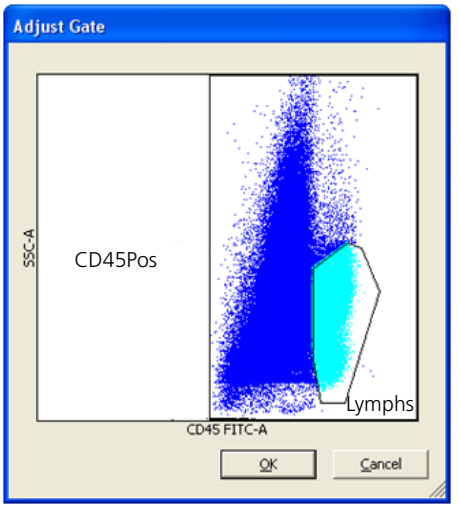
Auto gating



No manual gate adjustment is necessary.

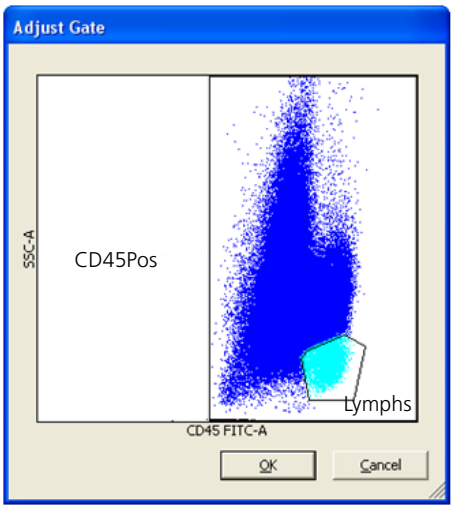
Step 4, plot 1

Auto gating



The Lymphs gate includes too many non-lymphocyte events.

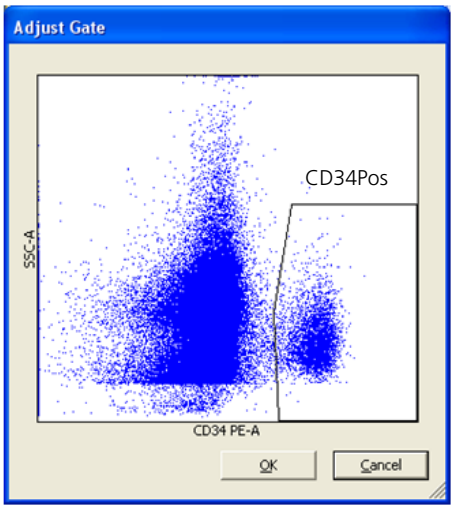
Manual gating



Manually adjust the Lymphs gate so that it includes only the lymphocytes.

Step 5, plot 2

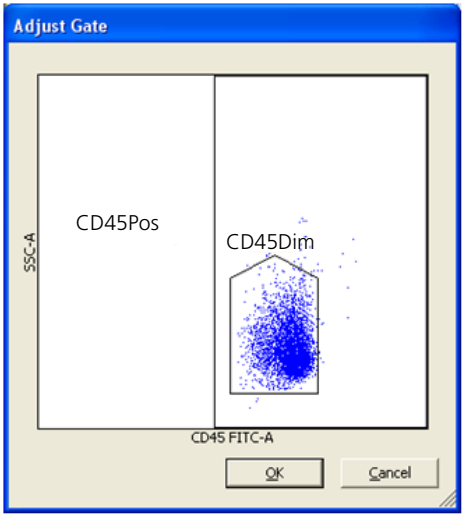
Auto gating



No manual gate adjustment is necessary.

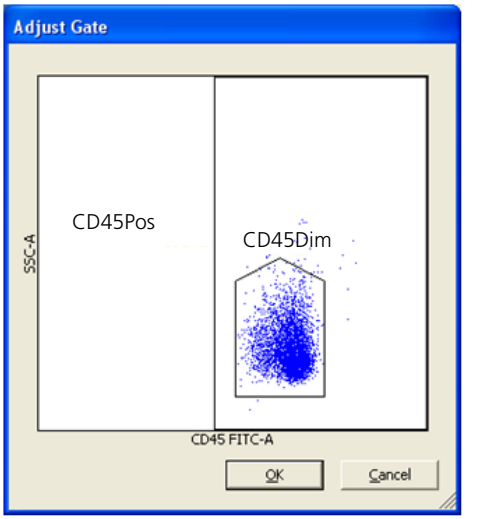
Step 6, plot 3

Auto gating



The CD45Dim gate does not completely enclose the CD45Dim population.

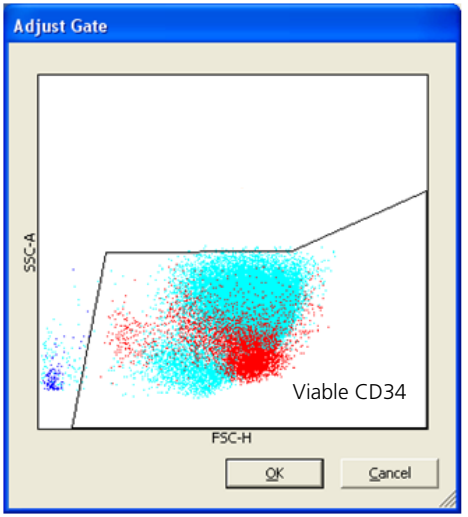
Manual gating



Manually adjust the CD45Dim gate so that it includes all of the CD45Dim events.

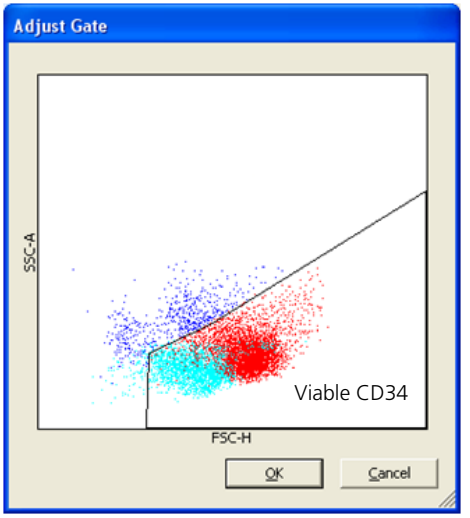
Step 7, plot 4

Auto gating



The Viable CD34 gate is dependent on the correct gating of the viable lymphocytes in plot 1. Always adjust the Viable CD34 gate if the Lymphs gate was adjusted.

Manual gating

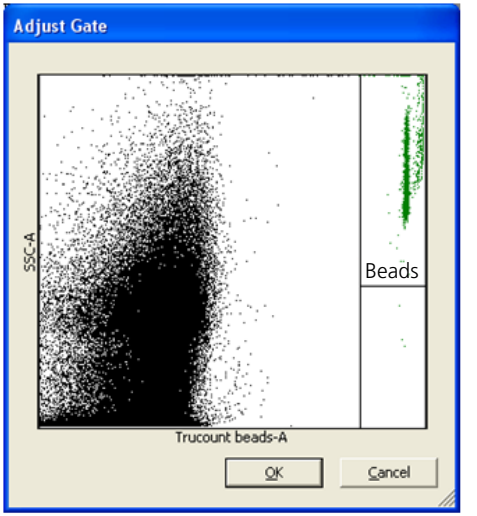


Manually adjust the Viable CD34 gate so that it includes the left edge and top third of the viable lymphocytes. Cells with low FSC and higher SSC are not viable as defined by scatter.

Note that the two plots look very different. Changes made to previous gates affect how populations appear in subsequent plots.

Step 8, plot 5

Auto gating



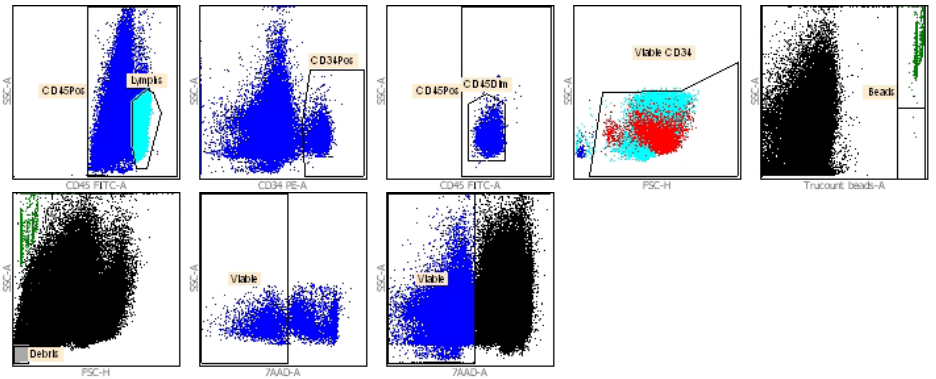
No manual gate adjustment is necessary.

Lab Report for auto gating

Specimen			
ALP004-BD1			
Director: BD		Panel:	BD Stem Cell + 7AAD
		Acquired:	7/1/2008 5:37:59 PM
		Analyzed:	7/16/2008 2:47:53 PM
		TruC Lot ID:	12007
		Bead/Pellet:	52000
		Status:	OK
		Operator:	PAL
		Reviewer:	
		Results:	16072008.csv
Dilution Factor: 11	Column #2:		Column #3:

BD FACSCanto II V96322014 BD FACSCanto v.2.4.3155.23769

BD Stem Cell + 7AAD Total Events: 295630



ALP004-BD1005.001.fcs Kit Lot ID: IUO24371

CD34+ Viable Abs Cnt (cells/µl)	4787.34
CD45+ Viable Abs Cnt (cells/µl)	83522.63
CD34+ Viable Events	4322
CD45+ Viable Events	75404
Bead Events	5164
Viable CD34+ % Viable CD45+	5.73
CD34 Viability (%)	48.72
Viable CD34+ CV (%)	1.52

QC Messages
Inspect all dot plots.

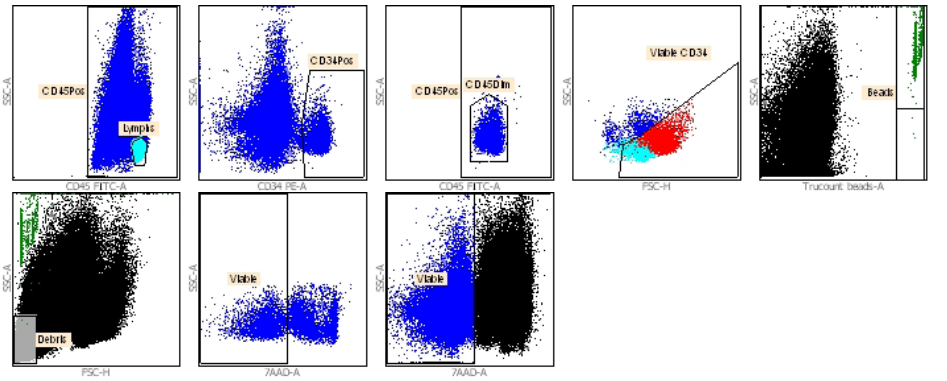
Comments

Lab Report for manual gating

Specimen			
ALP004-BD1			
Director: BD		Panel:	BD Stem Cell + 7AAD
		Acquired:	7/1/2008 5:37:59 PM
		Analyzed:	7/16/2008 2:53:35 PM
		TruC Lot ID:	12007
		Bead/Pellet:	52000
		Status:	OK
		Operator:	PAL
		Reviewer:	
		Results:	16072008.csv
Dilution Factor: 11	Column #2:		Column #3:

BD FACSCanto II V96322014 BD FACSCanto v.2.4.3155.23769

BD Stem Cell + 7AAD Total Events: 295630



ALP004-BD1005.001.fcs Kit Lot ID: IUO24371

CD34+ Viable Abs Cnt (cells/µl)	4039.67
CD45+ Viable Abs Cnt (cells/µl)	59838.46
CD34+ Viable Events	3647
CD45+ Viable Events	54022
Bead Events	5164
Viable CD34+ % Viable CD45+	6.75
CD34 Viability (%)	41.90
Viable CD34+ CV (%)	1.66

QC Messages

Manual Gate is in effect.
Inspect all dot plots.

Comments

Bone marrow sample (fresh)

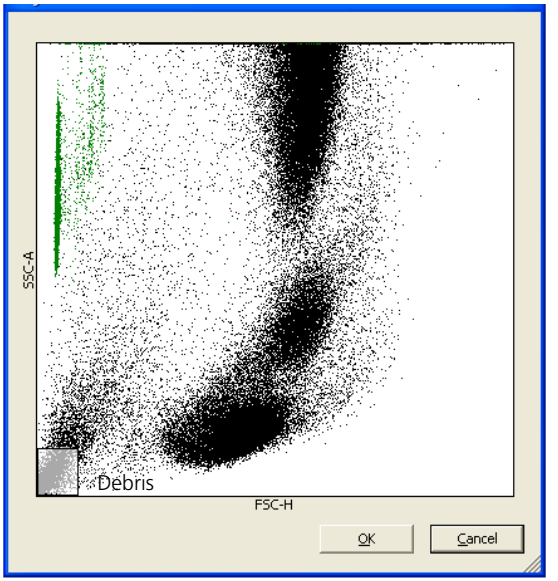
About this topic

This topic shows an example of how BD FACSCanto clinical software processes a fresh bone marrow sample stained using the BD Stem Cell Enumeration kit and analyzed on a BD FACSCanto II flow cytometer. Gates shown are auto-gates set by the software. Manual gate adjustment was not necessary. See the Gating guideline (page 46) for more information.

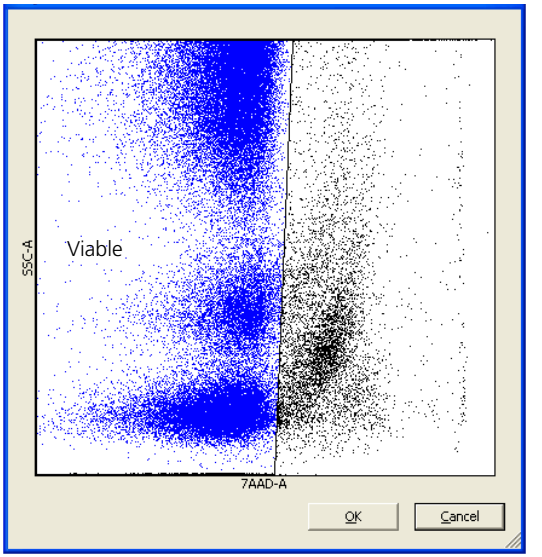
How to use this information

Use this information to review your data plots, verify the placement and boundaries of each gate, and adjust the gates as necessary.

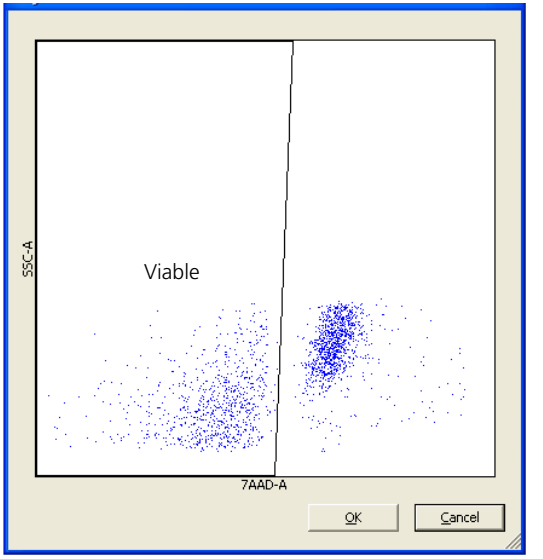
Step 1, plot 6



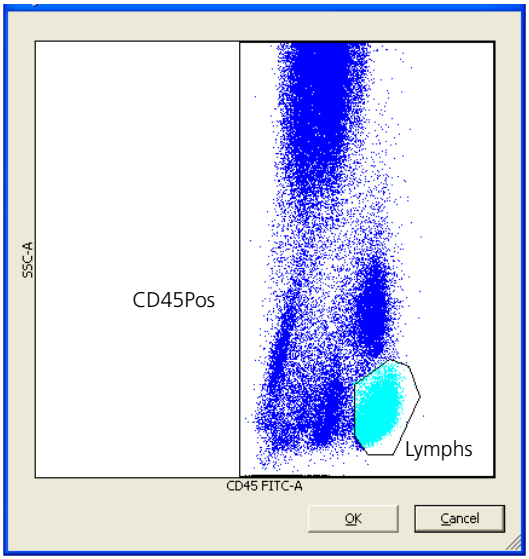
Step 2, plot 8



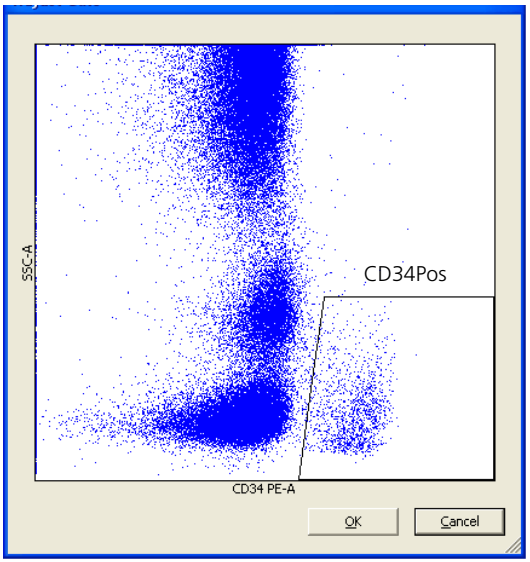
Step 3, plot 7



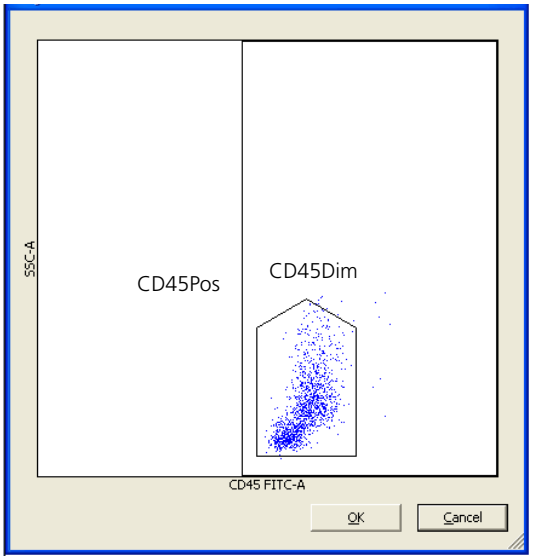
Step 4, plot 1



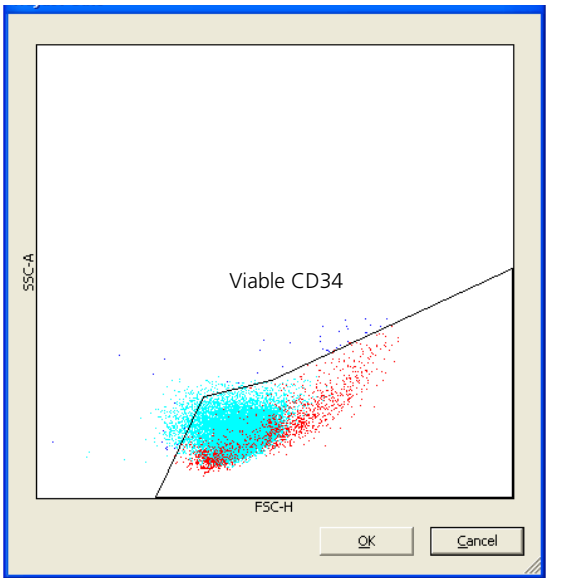
Step 5, plot 2



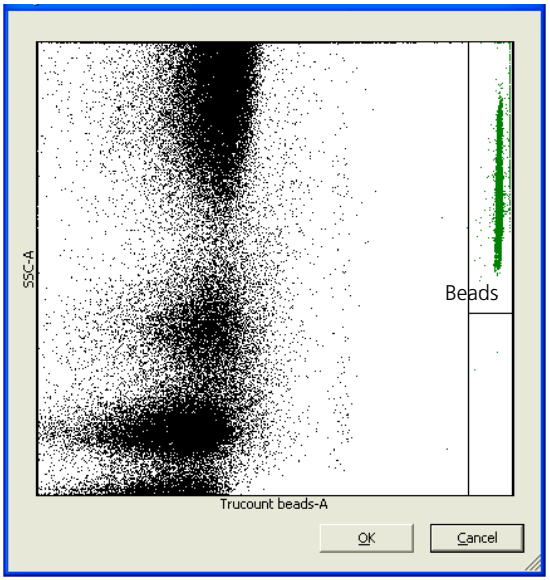
Step 6, plot 3



Step 7, plot 4



Step 8, plot 5



Bone marrow sample (thawed)

About this topic

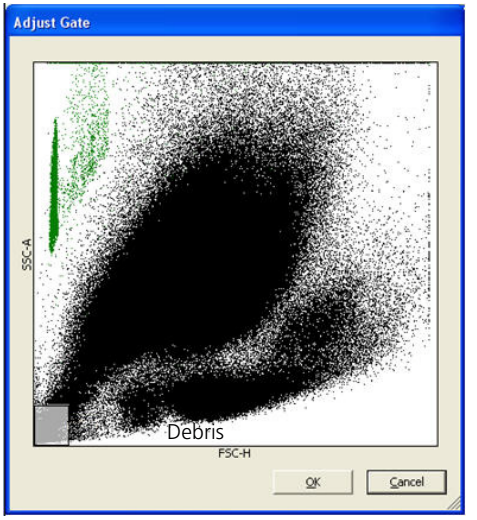
This topic shows an example of how BD FACSCanto clinical software processes a thawed bone marrow sample stained using the BD Stem Cell Enumeration kit and analyzed on a BD FACSCanto II flow cytometer (plots are labeled Auto gating). A second set of plots (labeled Manual gating) shows an example of how to manually adjust each gate. See the Gating guideline (page 46) for more information.

How to use this information

Use this information to review your data plots, verify the placement and boundaries of each gate, and adjust the gates as necessary.

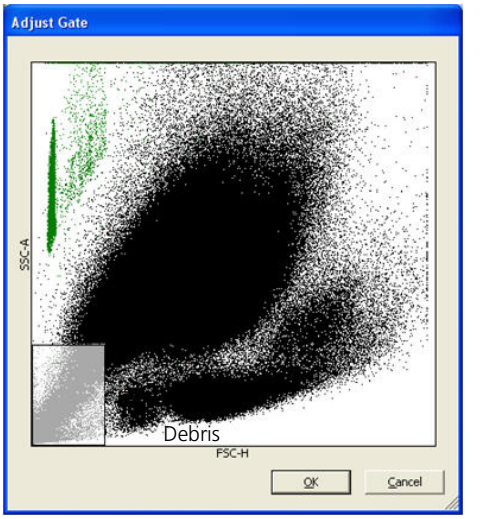
Step 1, plot 6

Auto gating



The Debris gate is too small.

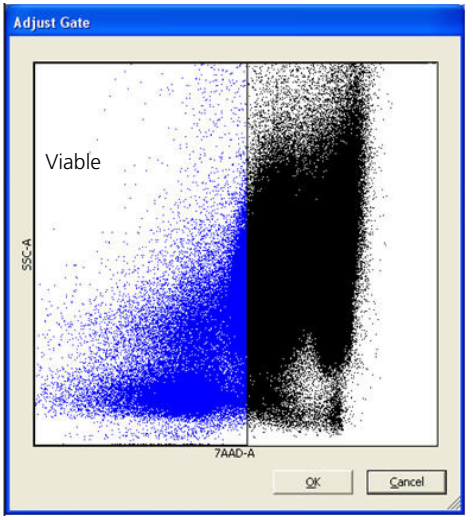
Manual gating



Increase the Debris gate. Subsequent plots will show less debris and populations of interest will be more visible.

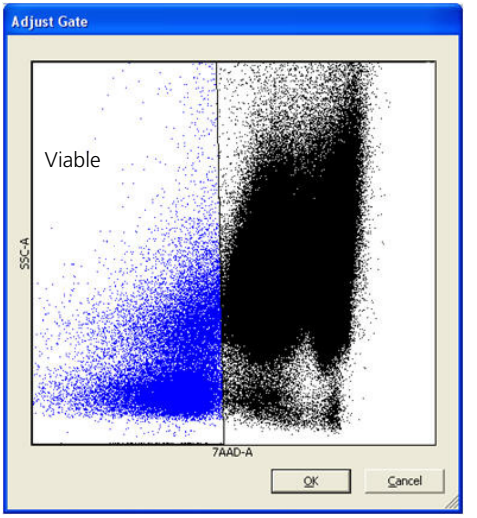
Step 2, plot 8

Auto gating



The Viable gate is too wide.

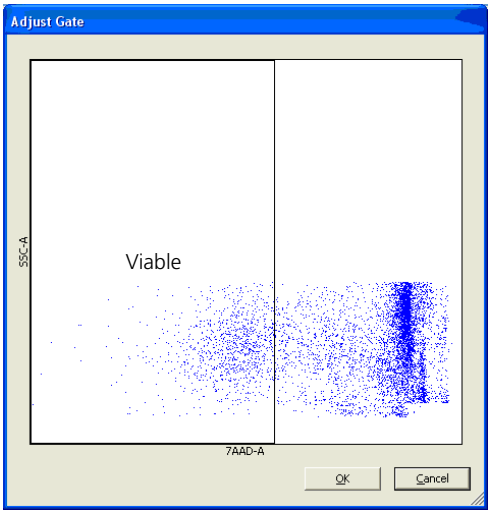
Manual gating



Manually adjust the Viable gate so that it includes only the lymphocytes and monocytes.

Step 3, plot 7

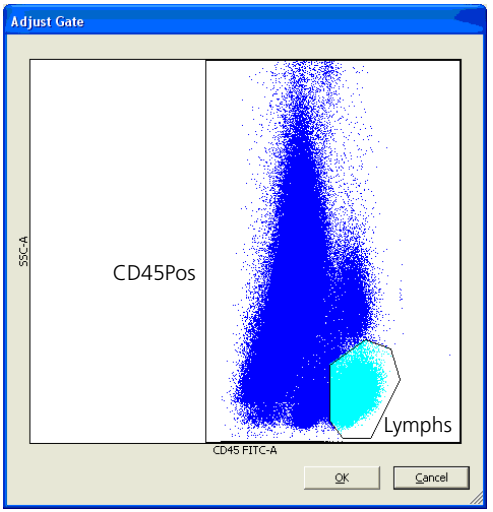
Auto gating



No manual gate adjustment is necessary.

Step 4, plot 1

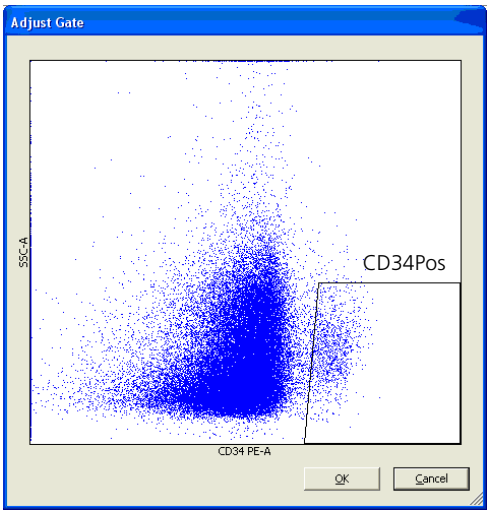
Auto gating



No manual gate adjustment is necessary.

Step 5, plot 2

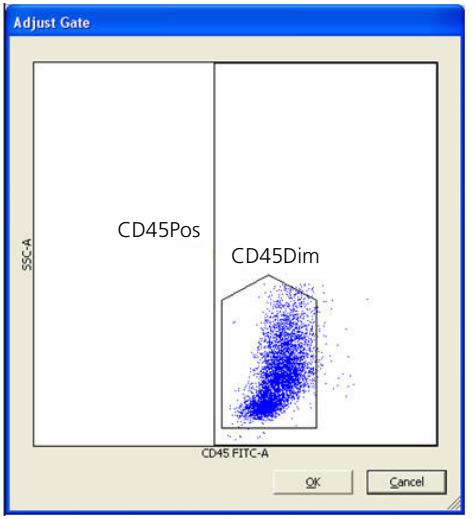
Auto gating



No manual gate adjustment is necessary.

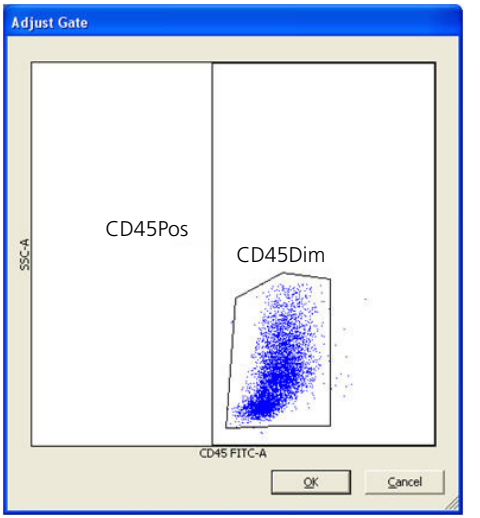
Step 6, plot 3

Auto gating



The right edge of the CD45Dim gate does not include all of the cells in the cluster.

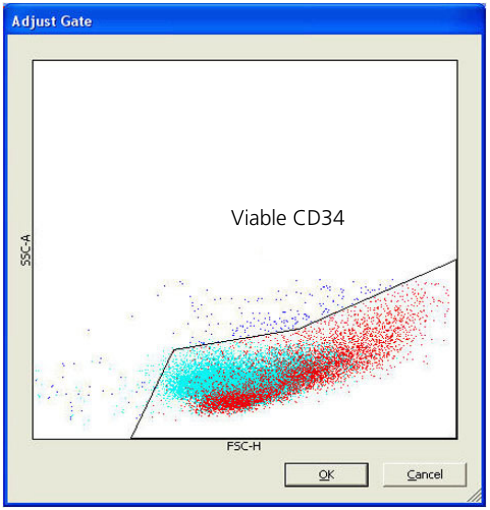
Manual gating



Manually adjust the CD45Dim gate so that it includes the cell cluster, but not the brighter lymphocytes.

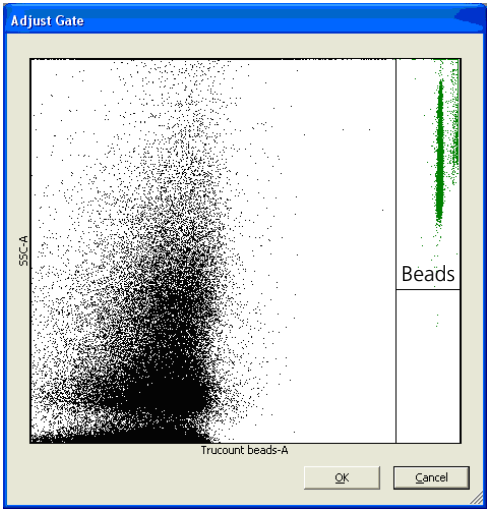
Step 7, plot 4

Auto gating



Step 8, plot 5

Auto gating



No manual gate adjustment is necessary.

Cord blood sample (fresh)

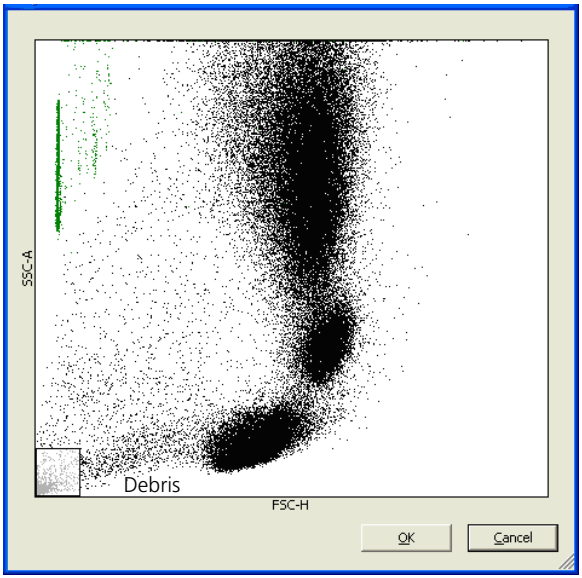
About this topic

This topic shows an example of how BD FACSCanto clinical software processes a fresh cord blood sample stained using the BD Stem Cell Enumeration kit and analyzed on a BD FACSCanto II flow cytometer. Gates shown are auto-gates set by the software. Manual gate adjustment was not necessary. See the Gating guideline (page 46) for more information.

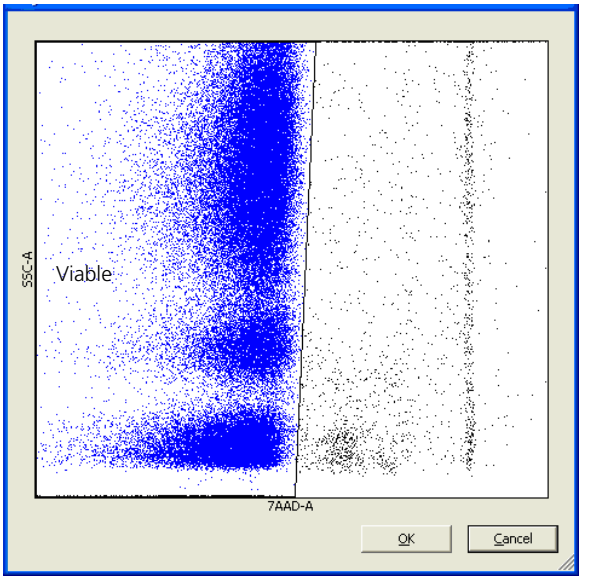
How to use this information

Use this information to review your data plots, verify the placement and boundaries of each gate, and adjust the gates as necessary.

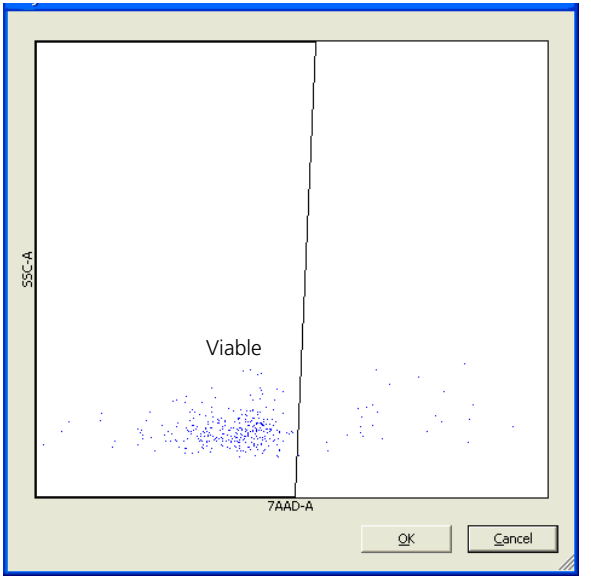
Step 1, plot 6



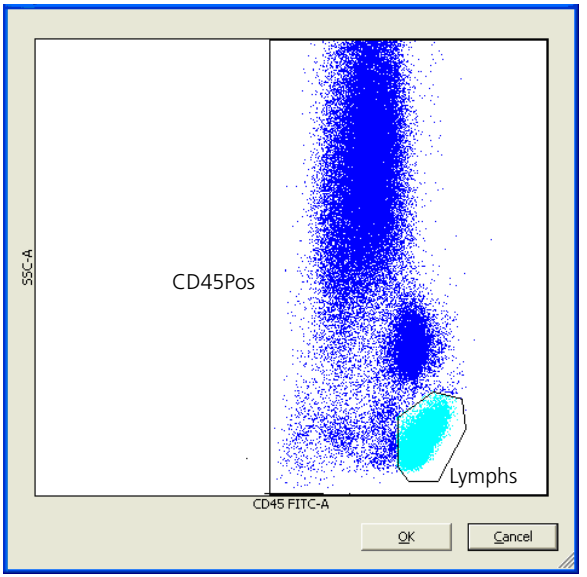
Step 2, plot 8



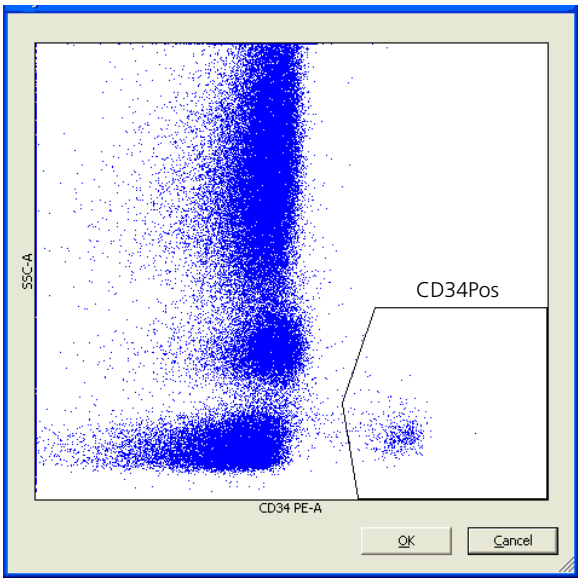
Step 3, plot 7



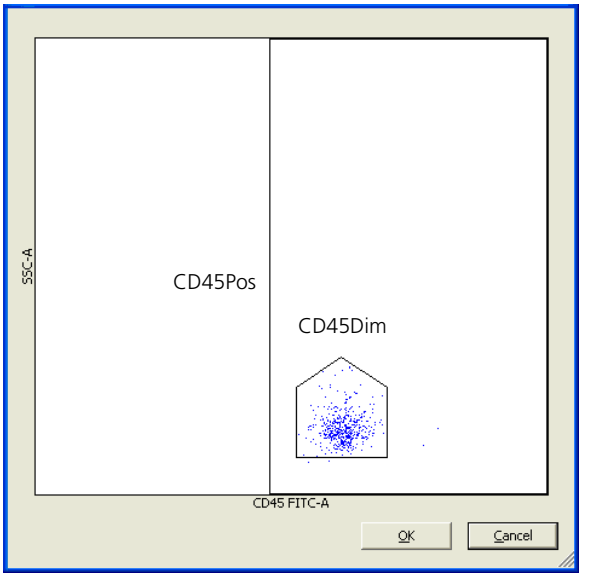
Step 4, plot 1



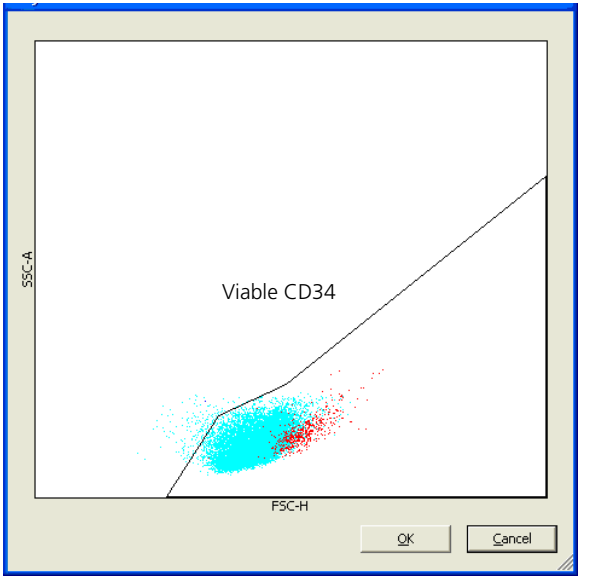
Step 5, plot 2



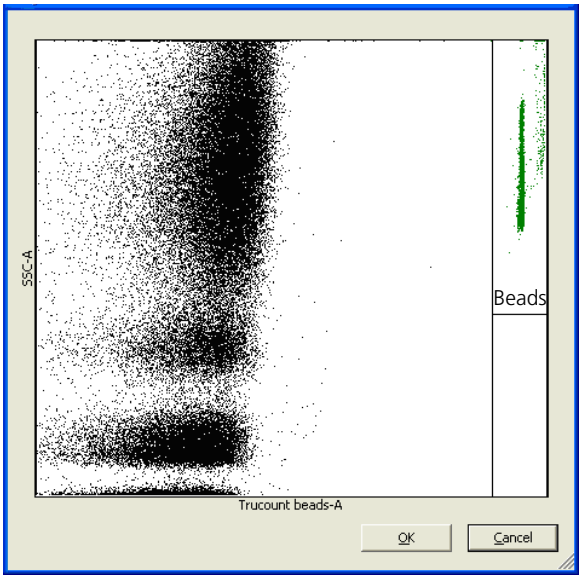
Step 6, plot 3



Step 7, plot 4



Step 8, plot 5



Cord blood sample (thawed)

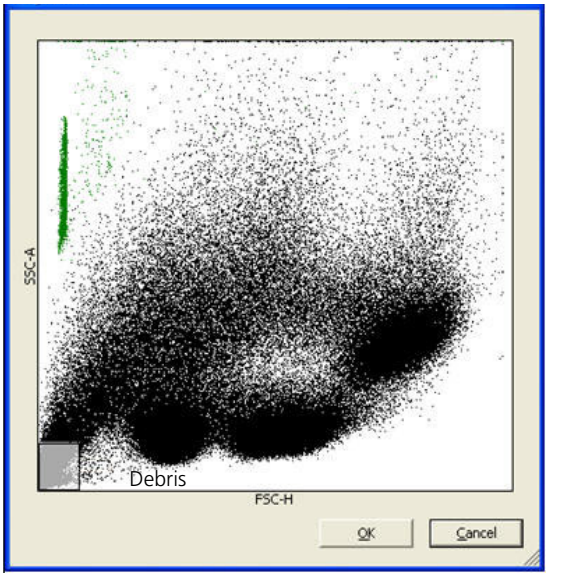
About this topic

This topic shows an example of how BD FACSCanto clinical software processes a thawed cord blood sample stained using the BD Stem Cell Enumeration kit and analyzed using a BD FACSCanto II flow cytometer. Gates shown are auto-gates set by the software. Manual gate adjustment was not necessary. See the Gating guideline (page 46) for more information.

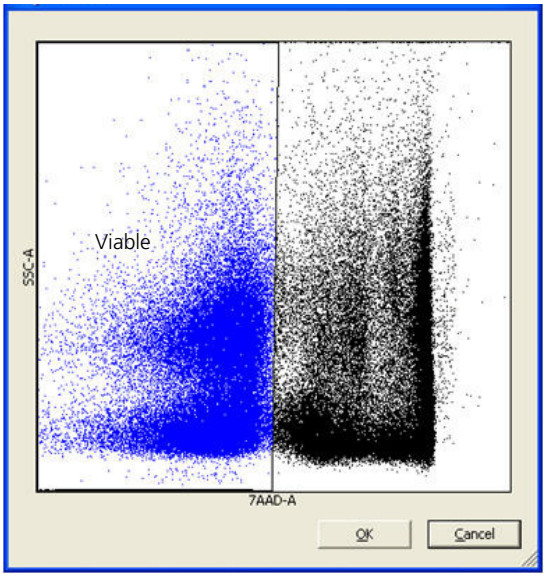
How to use this information

Use this information to review your data plots, verify the placement and boundaries of each gate, and adjust the gates as necessary.

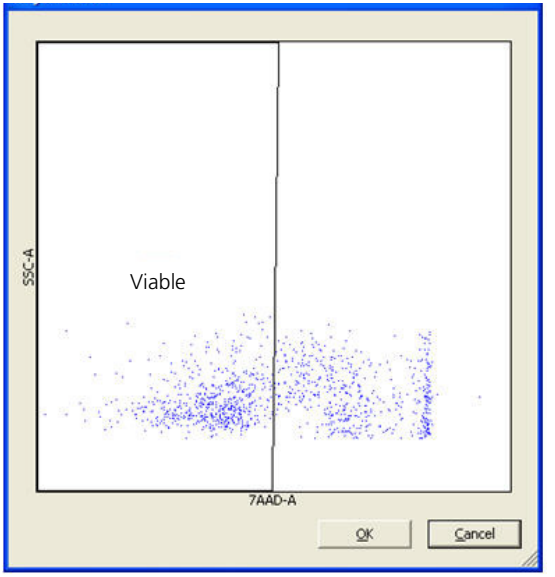
Step 1, plot 6



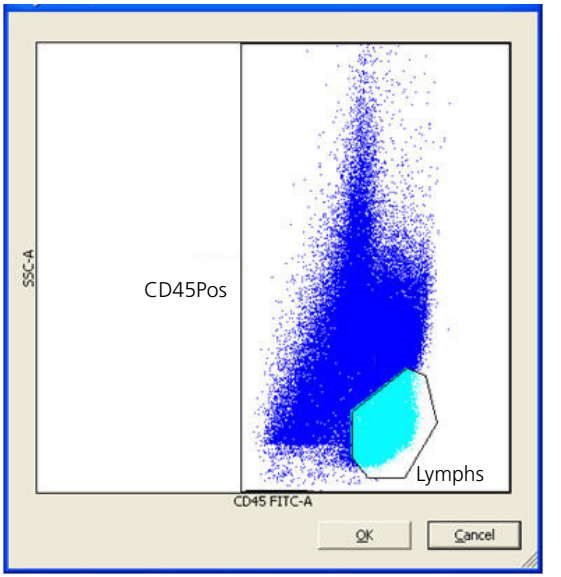
Step 2, plot 8



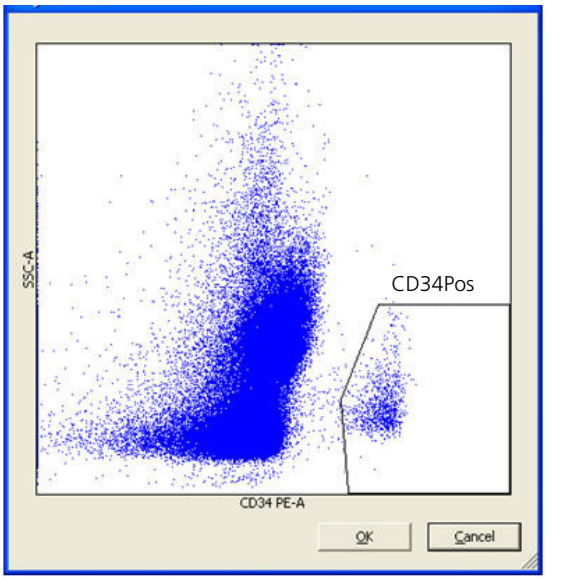
Step 3, plot 7



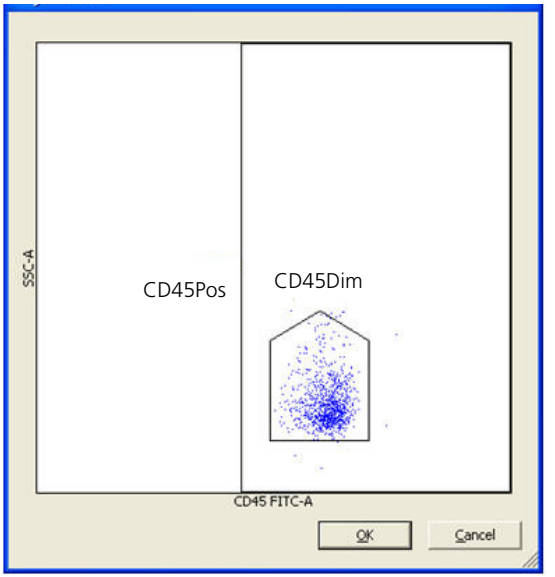
Step 4, plot 1



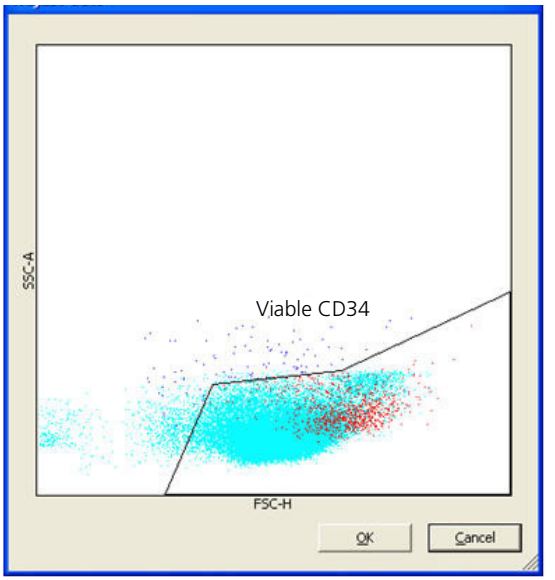
Step 5, plot 2



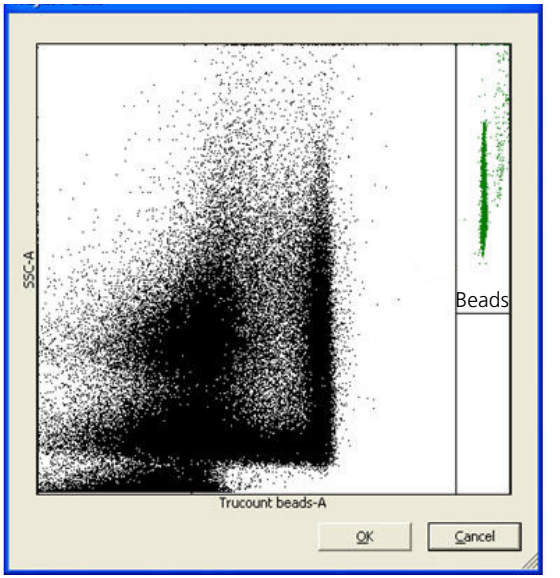
Step 6, plot 3



Step 7, plot 4



Step 8, plot 5



7

Reviewing reports

This chapter covers the following topics:

Reviewing the Cytometer Setup Report

About this topic

This topic describes the information found on the Cytometer Setup Report.

Cytometer Setup Report

The Cytometer Setup Report is generated after running cytometer setup.

Cytometer Setup Report

1

Cytometer:	BD FACSCanto II	Institution:	
Serial Number:	V96300098	Director:	
Software:	BD FACSCanto v.2.4.3155.23769	Operator:	USERS
Date:	07/07/2007 3:08:15 AM	Overall Result:	PASS

2

Setup Beads

Bead Product: BD FACS 7-Color Setup Beads, Catalog Number: 335775

Lot Information: Lot ID 54321, Exp.: 2007-11-28

3

Detectors

	a	b	c	d	e	f	g
Detector	Laser	FL Target	Voltage	ΔVoltage	Sensitivity	Spec	P/F*
FSC	Blue	456	201	0	NA	NA	PASS
SSC	Blue	555	399	-1	NA	NA	PASS
FITC	Blue	460	471	0	29	15	PASS
PE	Blue	402	449	0	167	80	PASS
PerCP	Blue	490	677	0	10	9	PASS
PerCP-Cy5.5	Blue	480	624	0	28	25	PASS
PE-Cy7	Blue	415	553	0	311	120	PASS
APC	Red	415	599	0	110	40	PASS
APC-Cy7	Red	411	535	0	37	16	PASS

*ΔVoltage (change from previous setup): < 50 volts. Sensitivity: > Spec

4

Compensation

	Fluorophores (% spectral overlap)				PASS	spec: all values ≤ 100%	
Detector	FITC	PE	PerCP	PerCP-Cy5.5	PE-Cy7	APC	APC-Cy7
FITC	100.00	1.86	0.00	0.00	0.24	0.30	0.04
PE	11.74	100.00	0.04	0.01	2.07	0.22	0.01
PerCP	3.59	34.60	100.00	100.00	22.91	1.62	0.95
PerCP-Cy5.5	3.59	34.60	100.00	100.00	22.91	1.62	0.95
PE-Cy7	0.18	1.69	3.83	11.71	100.00	0.17	3.62
APC	0.02	0.17	5.16	4.45	0.00	100.00	21.45
APC-Cy7	0.00	0.03	0.45	8.27	6.02	3.23	100.00

5

Lasers

Laser	Power (mW)	Spec. (mW)	P/F	Current (A)
Blue	20.02	16.5-24.76	PASS	1.57
Red	27.19	22.73-34.09	PASS	NA

6

Fluidics

FACSFlow Pressure

Pressure	4.5 PSI
Spec	4.5 +/- 0.1 PSI
P/F	PASS

Sample Pressure (PSI)

High	Medium	Low
1.5	0.8	0.3

7

Comments

8

Reviewed By: _____

Report contents

The following table describes the information shown in the Cytometer Setup Report. The numbers correspond with those shown in the example report.

No.	Name	Function
1	Report Header	Contains the cytometer name and serial number, software version, date and time of setup, institution, director, operator, and overall result (PASS or FAIL).
2	Setup Beads	Lists the bead product used, catalog number, lot ID, and expiration date.
3	Detectors	Provides the following information: • **Laser (a).** Indicates which laser excited the stained particle to emit light collected for that detector. • **FL Target (b).** Fluorescence target value in log form. The software adjusts the voltage so that the setup bead is at the target value. For more information about the FL Target, see the beads package insert. • **Voltage (c).** Indicates the voltage required to place the beads at the fluorescence target values (b). • **ΔVoltage (d)** indicates the change in volts from the last setup. The difference between the two values should be less than 50 volts. A difference of less than 50 will pass (g). A difference of 50 or greater will fail. • **Sensitivity (e).** Measure of the cytometer's ability to resolve dimly stained cells. The measurement includes contributions from efficiency of photon collection, background signal, and intrinsic brightness of each fluorophore. • **Spec (f).** A sensitivity value greater than the Spec means that the detector passes the sensitivity specification (g). • **P/F (g).** Indicates whether the Sensitivity (e) or ΔVoltage (d) passed or failed. A failure in either category will cause an overall Fail (g) for that detector.
4	Compensation	Displays spectral overlap values calculated during setup for the current voltages. Values ≤100% will pass. Values >100% will fail.

No.	Name	Function
5	Lasers	Provides information about each laser and whether or not it passes the power specifications (in milliwatts) determined by BD Biosciences. The laser current (measured in amperes) is also provided.
6	Fluidics	Shows whether or not the sheath pressure meets BD Biosciences determined specifications. It also shows sample pressure voltage for low, medium, and high flow rates, which is useful when troubleshooting.
7	Comments	Provides an area to enter additional information.
8	Reviewer	Provides a space for the reviewer to sign the report.

Troubleshooting information

For help with out of range values on the Cytometer Setup Report, see the *BD FACSCanto Software Reference Manual* or the *BD FACS 7-color setup beads* package insert.

More information

- Setting up the cytometer (page 28)

Reviewing the Application Setup Report

About this topic

The Application Setup Report shows assay-specific cytometer settings. The software uses these settings if you choose not to optimize with process controls.

Application Setup Report

The Application Setup Report is generated after running cytometer setup. The following is an example setup report.

1

Application Setup Report
BD Stem Cell

Cytometer:	BD FACSCanto II	Institution:	
Serial Number:	V96300098	Director:	
Software:	BD FACSCanto v2.4.3155.23769	Operator:	USERS
Date:	7/16/2007 04:37:00 AM		

2 **Cytometer Setup**

Cytometer Setup Report: 7/16/2007 04:37:00 AM, Overall Result: PASS

3 Bead Product: BD FACS 7-Color Setup Beads, Catalog Number: 335775
Lot Information: Lot ID 76697, Exp.: 2007-09-30

4 **Detectors**

Detector	a Laser	b Voltage
FSC	Blue	317
SSC	Blue	407
FITC	Blue	357
PE	Blue	396
7AAD	Blue	438
Trucount beads	Red	516

5 **Compensation**

	Fluorophores (%spectral overlap)			
Detector	FITC	PE	7AAD	Trucount beads
FITC	100.00	0.48	0.00	0.01
PE	24.67	100.00	5.50 c	0.01
7AAD	2.40	10.81	100.00	0.51
Trucount beads	0.00	0.09	10.09	100.00

6 **Threshold** (Operator: And)

FITC	400

7 **Comments**

8 Reviewed By: _____

Report contents

The following table describes the information shown in the Application Setup Report. The numbers correspond with those shown in the example report.

No.	Name	Function
1	Report Header	Contains the cytometer name and serial number, software version, date and time of run, institution, director, and operator.
2	Setup Results	Lists the date and time cytometer setup was performed and the setup result (PASS or FAIL).
3	Setup Beads	Lists the bead product used, catalog number, lot ID, and expiration date.
4	Detectors	Provides the following information: • **Laser (a).** Indicates which laser excited the stained particle to emit light collected for that detector. • **Voltage (b).** Indicates the voltage required to place the beads at the fluorescence target values.
5	Compensation	Displays spectral overlap values calculated during setup for the current voltages. Values ≤100% will pass. Values >100% will fail. **PE–%7AAD value (c).** A value of –0.5% to 10.5% indicates a successful setup optimization.
6	Threshold	Indicates the parameter(s) and value(s) used as the threshold during optimization. It also shows the logical operator in effect for the threshold(s). Logical operator choices are: • OR (only one threshold) • AND (all selected thresholds)
7	Comments	Provides an area to enter additional information.
8	Reviewer	Provides a space for the reviewer to sign the report.

More information

• Setting up the application (page 31)

Reviewing the Lab Report

About this topic The Lab Report shows sample plots, analyzed data, and QC messages.

Lab Report A Lab Report is generated after each sample.

1

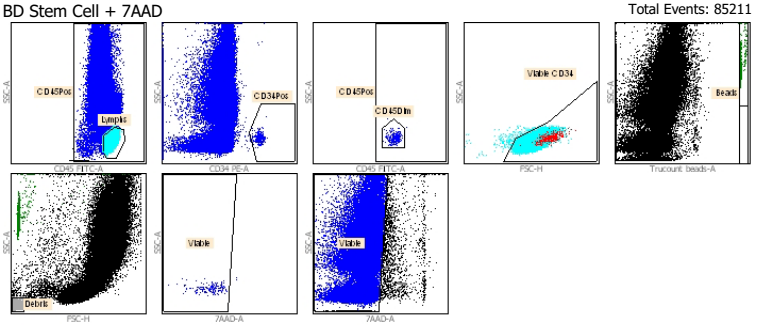
Specimen
ALP16-BD1
Director: BD

Panel:	BD Stem Cell + 7AAD
Acquired:	7/1/2008 10:34:08 AM
Analyzed:	7/16/2008 6:49:06 PM
TruC Lot ID:	12007
Bead/Pellet:	52000
Status:	OK
Operator:	PAL
Reviewer:	
Results:	01072008.csv

2 Dilution Factor: 4.0 Column #2: Column #3:

3 BD FACSCanto II V96322004 BD FACSCanto v.2.4.3155.23769

4 BD Stem Cell + 7AAD Total Events: 85211



5 ALP16-BD1003.001.fcs Kit Lot ID: 1UO24371

6

CD34+ Viable Abs Cnt (cells/µl)	481.10
CD45+ Viable Abs Cnt (cells/µl)	123837.80
CD34+ Viable Events	303
CD45+ Viable Events	77994
Bead Events	1310
Viable CD34+ % Viable CD45+	0.39
CD34 Viability (%)	99.34
Viable CD34+ CV (%)	5.74

7 **QC Messages**
Inspect all dot plots.

8 **Comments**

Report contents

The following table describes the information shown in the BD Stem Cell Lab Report. The numbers correspond with those shown in the example report.

No.	Name	Function
1	Report Header	Contains information about the lab, reagents, assay, and sample, and acquisition and analysis dates and times.
2	Column Titles	Shows values entered into the panel-specific columns of the Worklist.
3	Cytometer and Software Information	Lists the cytometer name and serial number and the software name and version number.
4	Plots	Shows the plots and the analyzed data, as well as the total number of events collected. **Note:** The two viability plots are omitted from the report when running process controls.
5	Sample File Name and Kit Lot #	Lists the sample file name and the lot number of the BD Stem Cell Enumeration kit.
6	Results	Reports the results of the analysis for each tube. A lab manager can choose which results to display and change the alarm ranges (see the *BD FACSCanto II Software Reference Manual* or the *BD FACSCanto II Instructions for Use*).
7	QC Messages	This section shows: • Quality control values selected by the lab manager • Message indicating a failure occurred • Message when one or more results are outside the alarm range
8	Comments	Provides an area to enter additional information.

More information

• Troubleshooting (page 93)

8

Troubleshooting

This chapter covers the following topics:

Contact and troubleshooting information

About this topic

This topic offers suggestions to help you troubleshoot your experiments and lists contact information if additional assistance is required.

How to get support

Use the following information to contact support.

- For support from within the US, call (877) 232-8995.
- For support from within Canada, call (888) 259-0187.
- For support outside the US and Canada, contact your local BD representative or distributor.
- See our website (bdbiosciences.com) for up-to-date contact information.

Spillover failure

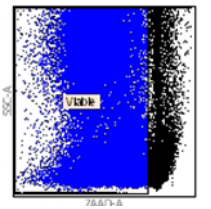
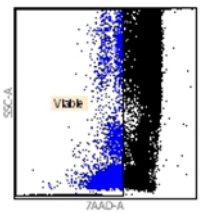
QC message	Possible cause	Recommended solution
Spillover failure.	• Did not add the 7-AAD to the tube. • Did not use BD Stem Cell controls to perform setup. • Did not add the CD45/CD34 reagent to the tube.	Re-stain and run the setup and optimization tubes. Check the cytometer fluidics.

Worklist will not run

QC message	Possible cause	Recommended solution
Worklist will not run.	Standard Setup was run after Application Setup.	1. Re-stain the Setup and Optimization tubes. 2. Re-run Standard Setup followed by Application Setup.

No results reported

QC message	Possible cause	Recommended solution
Lymph gate failure.	Insufficient number of lymphs in the sample.[a]	Manually re-gate the sample.
	Unresolved lymphocyte population.	Manually re-gate the sample.
CD45 gate failure.	Insufficient number of CD45 cells in the sample.[a]	Manually re-gate the sample.
CD34Pos gate failure.	Insufficient number of CD34Pos cells in the sample.[a]	Manually re-gate the sample.
Viable CD34 gate failure.	Insufficient number of Viable CD34 cells in the sample.[a]	Manually re-gate the sample.

QC message	Possible cause	Recommended solution
CD34 gate failure.	Insufficient number of CD34 cells in the sample.[a]	Manually re-gate the sample.
Viability gate failure.	Insufficient number of viable cells in the sample because all cells are dead. 	Repeat staining, then acquire the newly stained sample.
	Insufficient number of viable cells in the sample because 7-AAD was added to the process control. 	1. Repeat staining following the *BD Stem Cell Enumeration kit* package insert. (Do not add 7-AAD to the process controls.) 2. Acquire the newly stained process controls.

a. Check acquisition targets for set values. See Viewing acquisition targets (page 24).

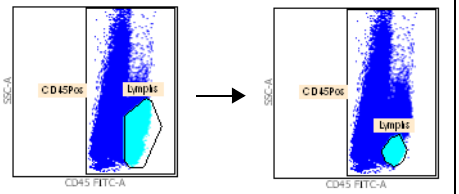
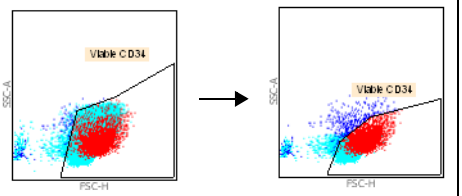
Incomplete results reported

QC message	Possible cause	Recommended solution
No beads detected.	• A BD Trucount tube was not used. • The pellet was missing from the tube.	Repeat staining using a BD Trucount tube, then acquire the newly stained sample.
Less than 1,000 beads collected.[a]	A BD Trucount tube was not used.	Repeat staining using a BD Trucount tube, then acquire the newly stained sample.
Concentration of white blood cells is too high.	WBC concentration is >45,000 cells/µL.	Dilute the sample, then re-stain and acquire the newly stained sample.

a. Check acquisition targets for set values. See Viewing acquisition targets (page 24).

Gate location is suspect, but all results are reported

QC message	Possible cause	Recommended solution
Viability gate suspect.	Unusual gate location.	Manually re-gate the sample.
Beads gate suspect.	Unusual gate location.	Manually re-gate the sample.
	• A BD Trucount tube was not used. • Pellet missing from tube.	Repeat staining using a BD Trucount tube, then acquire the newly stained sample.
CD34Pos gate suspect.	Unusual gate location.	Manually re-gate the sample.

QC message	Possible cause	Recommended solution
CD45 gate suspect.	Unusual gate location.	Manually re-gate the sample.
CD45Dim gate suspect.	Unusual gate location.	Manually re-gate the sample.
Lymphs gate suspect.	Unusual gate location. (In this example, the gate includes monocytes.)	1. Manually re-gate the sample.  2. Manually adjust the CD34 gate. 

Insufficient number of events collected, but all results are reported

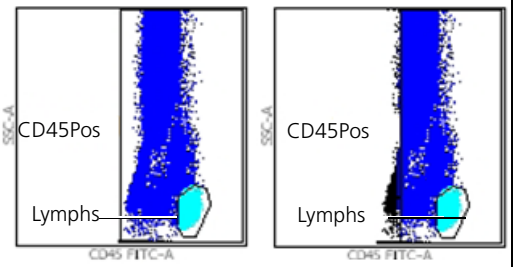
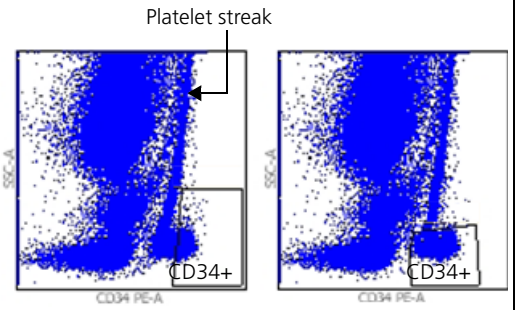
QC message	Possible cause	Recommended solution
Less than 100 Viable CD34 events collected.[a]	Low CD34 count. Acquisition timed out after 15 minutes.	1. Review the plots and report results. 2. Re-stain the sample, increase the stopping time, acquire the newly stained sample.
Less than user-requested[a] Viable CD34 events collected.	Low CD34 count. Acquisition timed out after 15 minutes.	1. Review plots and report results. 2. Re-stain the sample, increase the stopping time, acquire the newly stained sample.
Insufficient Viable CD45 events collected.	Insufficient number of viable cells in the sample. Acquisition timed out after 15 minutes.	Review plots and report results.
	Insufficient number of viable CD45 events in the sample. Acquisition timed out after 15 minutes.	• Review plots and report results. • Verify that the age of the specimen is <24 hours.
	The sample is too dilute.	Use a smaller dilution factor.
Less than user-requested Viable CD45 events collected.	Insufficient number of Viable CD45 events in the sample. Acquisition timed out after 15 minutes.	Review plots and report results.
	The sample is too dilute.	Use a smaller dilution factor.

a. Check acquisition targets for set values. See Viewing acquisition targets (page 24).

General warnings

QC message	Possible cause	Recommended solution
Cytometer settings were generated from a failed setup result.	Cytometer setup failed.	1. Re-run cytometer setup and verify that setup is successful. 2. Re-run the samples.
Setup optimization failed.	7-AAD was not added to the tube.	Add 7-AAD to the optimization tube and re-run setup.
	BD Stem Cell reagent was not added to the tube.	Re-stain the sample and acquire the newly stained sample.
	An incorrect control sample was used.	Re-stain and acquire controls.
One or more results are outside the alarm range.	The WBC concentration is >40,000 cells/µL.	Dilute the sample, then stain and acquire the newly stained sample.
Stem cell controls are out of range.	Cytometer setup failed.	Re-run cytometer setup and verify that setup is successful (Pass), then re-stain and re-run the controls.
	Pipetting error.	Re-stain and re-run the controls.
	Controls were not resuspended properly before pipetting.	Open a new tube of BD Stem Cell controls, re-stain, and re-run.
Research Use Only - Not for use in diagnostic or therapeutic procedures.	A BD FACSCanto cytometer and/or Loader was used to acquire samples.	Load tubes manually and acquire samples using a BD FACSCanto II flow cytometer.
Manual gate is in effect.	Gate(s) have been re-set manually.	NA. To reapply the gating algorithm, click **Autogate**.
Inspect all dot plots.	Dot plots must be reviewed to verify that gates were set correctly and cell populations appear as expected.	NA.

Heparin anticoagulant

Observation	Possible cause	Recommended solution
Platelet streak.	A sample was collected in heparin anticoagulant.	1. In plot 1, move the left boundary of the CD45Pos gate to the right to exclude the platelets.  Plot 1 before regating Plot 1 after regating 2. By adjusting the Lymphs gate in plot1, the platelet streak in plot 2 is partially removed.  Plot 2 before regating Plot 2 after regating

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