BD Stem Cell Enumeration Application Guide

For BD FACSLyric™ Flow Cytometers

23-21739-00
3/2021

Rx Only CE IVD

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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
23-21739-00	3/2021	Initial release

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Introduction

This chapter covers the following topics:

- About this guide (page 6)
- Technical support (page 7)

About this guide

What's in this guide

This guide covers the acquisition and analysis workflows for the BD® Stem Cell Enumeration 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™ Clinical 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® Stem Cell Enumeration 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™ Clinical System Instructions For Use* and the *BD FACSLyric™ Clinical Reference System*.

Contacting technical support

To contact technical support:

1. Go to bd.biosciences.com.
2. Select your region.
3. Click **Support.**
4. 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® Stem Cell Enumeration Assay

This chapter covers the following topics:

- About the assay (page 10)
- Assay workflow (page 11)
- Adding reagent information to the library (page 12)
- Adding 7-AAD to the reference settings (page 13)
- Updating reference settings (page 16)
- Running the assay (page 18)
- Reviewing the lab report (page 21)
- Gating strategy (page 25)
- Adjusting gates (page 30)
- Gating example: mobilized peripheral blood specimen (page 31)
- Gating example: leukapheresis specimen (fresh) (page 35)
- Gating example: leukapheresis specimen (post-thaw) (page 38)
- Gating example: bone marrow specimen (fresh) (page 41)
- Gating example: bone marrow specimen (post-thaw) (page 45)
- Gating example: cord blood specimen (fresh) (page 48)
- Gating example: cord blood specimen (post-thaw) (page 52)

About the assay

The BD® Stem Cell Enumeration Kit is used to enumerate viable dual-positive $CD45^+/CD34^+$ hematopoietic stem cell populations to determine absolute counts (cells/µL) of viable $CD34^+$ and the percentages of viable $CD45^+/CD34^+$ hematopoietic stem cells (%CD34).

Overview of the test

The reagent contains fluorochrome-conjugated antibodies that recognize CD45 and CD34. The CD45 antigen is present on all human leucocytes and is weakly expressed on hematopoietic progenitor cells. The CD34 antigen is present on immature hematopoietic precursor cells and all hematopoietic colony-forming cells in bone marrow and blood, including unipotent and pluripotent progenitor cells. The kit contains 7-amino actinomycin D (7-AAD) to assess the viability of the cells. Cells that are 7-AAD^+ are not viable. The kit also contains BD Trucount™ Tubes to determine absolute counts by comparing bead events to cell events.

Specimens are stained using the fluorochrome-conjugated antibodies and 7-AAD, and red blood cells are lysed. Stained samples are acquired on the flow cytometer and analyzed using BD FACSuite™ Clinical application.

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 12).
2	Add 7-AAD to the reference settings, as needed. See Adding 7-AAD to the reference settings (page 13).
3	Perform daily instrument Performance QC (PQC) using BD® CS&T Beads. See the *BD FACSLyric™ Clinical System Instructions For Use*.
4	Perform assay and tube settings setup for Stem Cell + 7-AAD using BD® CS&T Beads. See the *BD FACSLyric™ Clinical System Instructions For Use*.
5	Update reference settings, as needed. See Updating reference settings (page 16).
6	Prepare the process controls and patient specimens. See the *BD® Stem Cell Enumeration Kit* IFU for information.
7	Create worklist for the process controls and patient specimens. See Running the assay (page 18) or the *BD FACSLyric™ Clinical System Instructions For Use*.
8	Acquire samples. See Running the assay (page 18) or the *BD FACSLyric™ Clinical System Instructions For Use*.
9	Review the laboratory report. See Reviewing the lab report (page 21).
10	Adjust gates, if necessary. See Gating strategy (page 25).

Adding reagent information to the library

Before you begin, you must add the lot and expiration date for the BD® Stem Cell Reagent, 7-AAD, 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 Stem Cell Reagent or 7-AAD 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. Manually enter the **Lot ID**, found on the tube label.

6. Click the calendar icon and navigate through the calendar to select the expiration date, found on the tube label.

7. Select the **Current Lot** checkbox.

8. 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® Stem Cell Reagent and 7-AAD 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 Lot ID, expiration date, and Beads / Pellet in the appropriate field.

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

5. Ensure the Current Lot checkbox is checked.

6. Click **Done**.

Adding 7-AAD to the reference settings

Add 7-AAD to the Lyse Wash reference settings at the following times:

- Before performing the assay for the first time
- Whenever a new lot of BD® CS&T Beads is used without performing a Bead Lot Transfer
- When recommended by BD Service after cytometer maintenance or service is performed

Before you begin

- Prepare BD® CS&T Beads according to the IFU.
- If generating new SOVs using BD® FC Beads, prepare the beads according to the appropriate IFU.
- Prepare stained and unstained tubes for 7-AAD according to the *BD® Stem Cell Enumeration Kit* IFU.

Procedure

To add a fluorochrome:

1. On the navigation bar, click **Setup & QC**.

   The Setup & QC workspace opens.

2. In the **Setup & QC Options** panel, select **Add Fluorochromes** from the **Task** menu.
3. Select **Manual** or **Universal Loader** for the **Loading Option**.
4. If using the Loader, select **30 Tube Rack** or **40 Tube Rack** for the **Carrier Type**.
5. Select **Lyse Wash** for the **Reference Settings Name**.
6. In the **CS&T bead lot** field, select the appropriate bead lot.
7. Click **Start**.

   The Add Fluorochromes dialog opens.
8. Click **Add**.
9. Use the menus to add information about the control tubes.
   - In the **Fluorochrome** column, select 7-AAD.
   - In the **Control Type** column, select **FC**.
   - In the **Label** column, select **Generic**.
   - Do not enter information in the **Lot ID** column.
   - In the **Unstained** column, select **7-AAD - Unstained FC**.

     The system will prompt for a separate 7-AAD - Unstained tube.
10. Click **Next**.

    The **Load Tube** dialog opens. Follow the prompts of the software to load the appropriate tubes. If using the Loader, vortex the tubes immediately before loading the rack.

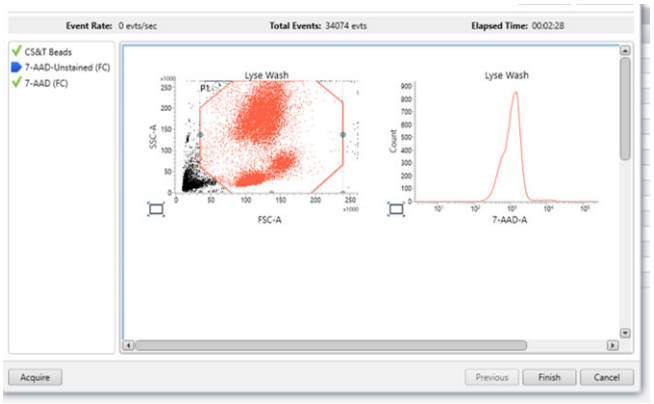
    When the BD® CS&T Beads are acquired, a green checkmark is displayed and the run pointer moves to the next tube.
11. When prompted, remove the BD® CS&T Beads tube, then load the 7-AAD - Unstained (FC) control tube onto the manual tube port.

    During acquisition, dot plots display the data.

12. When prompted, remove the 7-AAD - Unstained (FC) tube, then load the 7-AAD (FC) tube onto the manual tube port.

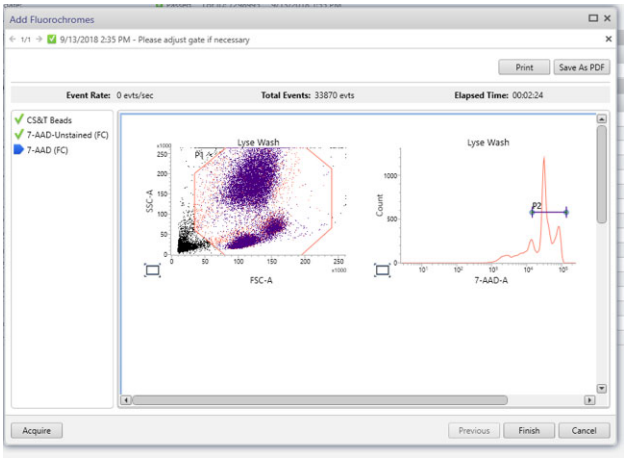
13. After acquiring both tubes, manually adjust the **P1** gate in the dot plot for the 7-AAD - Unstained (FC) tube to encompass all of the cells.

    Do not include the debris in the gate.



14. Set the run pointer to the 7-AAD (FC) sample and ensure that the P1 gate encompasses the same population of cells.

15. Adjust the **P2** gate in the histogram to encompass the major peaks as shown.



16. Click **Finish**.

    7-AAD is added to the existing tube settings, and the reference settings are updated to include the measured SOVs for the new fluorochrome.

Updating reference settings

Update reference settings every 60 days.

Before you begin

- Prepare BD® CS&T Beads according to the IFU.
- Prepare BD® FC Beads according to the appropriate IFU.
- Prepare stained and unstained tubes for 7-AAD according to the *BD® Stem Cell Enumeration Kit* IFU.

Note: If using the loader, ensure that all tubes are acquired within one hour, including 7-AAD. Take into account the time the tubes

will be on the loader before being acquired so that the last tube is acquired within one hour of staining.

Procedure

To update the reference settings:

1. On the navigation bar, click **Setup & QC**.

   The Setup & QC workspace opens.

2. In the **Setup & QC Options** panel, select **Update Reference Settings** from the **Task** menu.

3. Select **Manual** or **Universal Loader** for the **Loading Option**.

4. If using the Loader, select **30 Tube Rack** or **40 Tube Rack** for the **Carrier Type**.

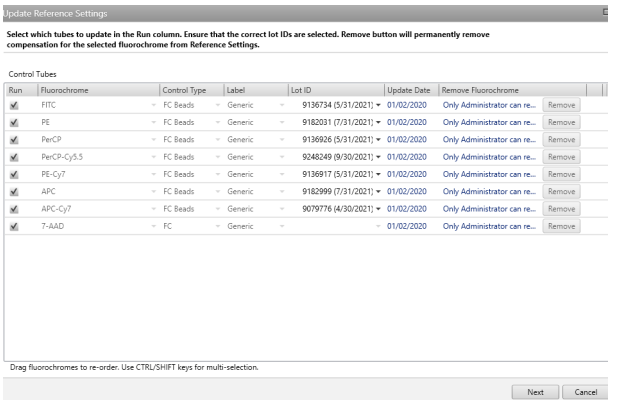
5. Select **Lyse Wash** for the **Reference Settings Name**.

6. In the **CS&T bead lot** field, select the appropriate bead lot.

7. Click **Start**.

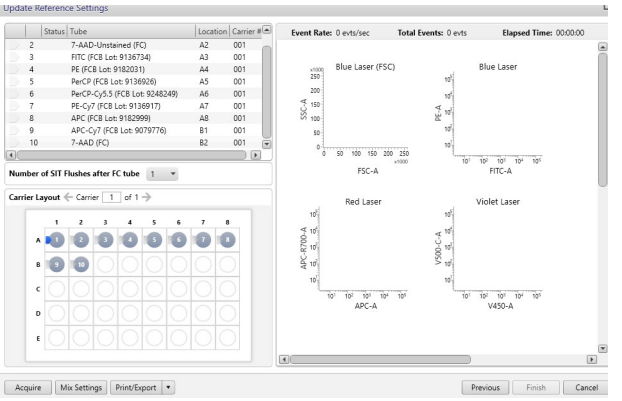
   The **Update Reference Settings** dialog opens.

8. In the **Run** column, select the fluorochromes you want to update.



9. Click **Next**.

10. If using the Loader, vortex each tube and place it in the rack according to the layout in the **Update Reference Settings** dialog.



11. Click **Acquire**.

    The spillover matrix for the selected fluorochromes is updated.

    For manual acquisition, follow the prompts in the software to load and 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™ Clinical System Instructions For Use* for more information.

Running the assay

To run the BD® Stem Cell Enumeration assay, you create a worklist and then acquire the samples.

Before you begin

1. Perform daily Performance QC (PQC) using BD® CS&T Beads.
2. Perform Assay/Tube Settings Setup for the BD® Stem Cell Enumeration assay.

We recommend selecting the Run Setup and Generate Reports checkboxes.

3. Ensure that the current lot and expiration date for BD® Stem Cell Reagent, 7-AAD, and BD Trucount™ Tubes are entered in the Library. See Adding reagent information to the library (page 12).

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 the appropriate task from the **Task** menu.

   Note: Create a Stem Cell Control task for each process control that you are running.

4. Manually enter the Sample ID for each task.

5. For each Stem Cell + 7-AAD sample, enter the following information, as needed:

   - Case Number
   - Pack Volume
   - Dilution Factor
   - Sample Name
   - Body Weight
   - Sample Type (from the menu)

6. Enter additional keywords if needed.

7. In the **Loading Options** section, select **Manual** from the **Loading Option** menu.

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

8. (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 Stem Cell Control samples and confirm that they pass before acquiring Stem Cell + 7-AAD patient samples.

2. In the **Worklist Controls** bar, select **Run from Pointer** from the **Run** menu.

3. Confirm the lot ID for the BD Trucount™ Tubes you are using.

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

5. 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™ Clinical System Instructions For Use* for more information.

   Acquisition will continue until each of the stopping criteria are met:

   – Viable CD45+ cells: 75,000 events collected
   – Viable CD34+ cells: 125 events collected
   – Trucount beads: 1,000 events collected

   If any of the stopping criteria are not met, acquisition will stop after 10 minutes. If any of the stopping criteria are not met and acquisition is timed out before 10 minutes, see Insufficient number of events collected, but all results are reported (page 62).

6. Examine each dot plot.

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

Reviewing the lab report

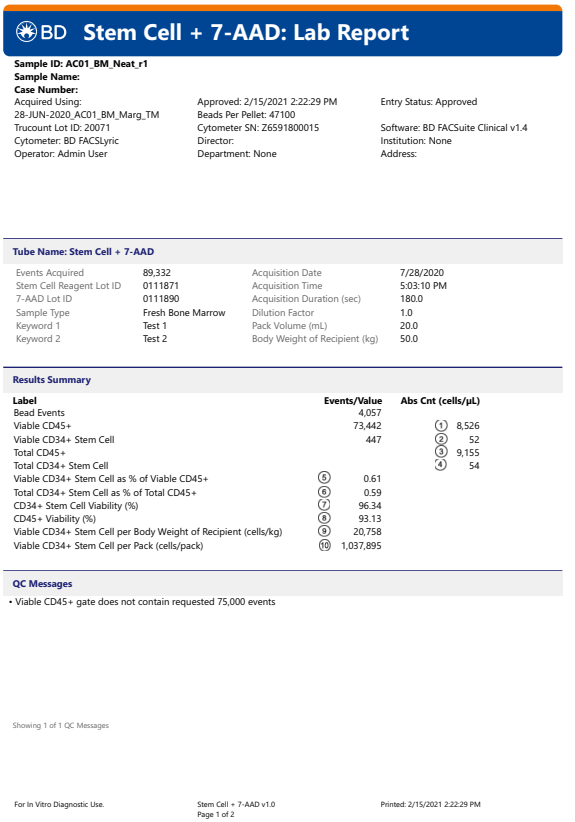
The Stem Cell + 7-AAD Lab Report contains assay and patient-specific information, reagent lot information, a results summary showing cell population statistics, QC messages, and dot plots with gates used to analyze the sample.

Viewing the lab report

1. Click the Lab Report tab to open the report.
2. Review page 1 of the lab report.
   a. Review the information about the cytometer, reagents, sample, and patient for accuracy.
   b. Review the Results Summary showing the cell population statistics.

      Most of the results reported are calculated values. Equations used to calculate the values are shown in the table that follows the lab report graphic in the next step.

c. Review any QC messages to address potential issues and determine whether they affect the results. See Troubleshooting (page 57).



BD Stem Cell + 7-AAD: Lab Report

Sample ID: AC01_BM_Neat_r1
Sample Name:
Case Number:

Acquired Using: 28-JUN-2020_AC01_BM_Marg_TM
Trucount Lot ID: 20071
Cytometer: BD FACSLyric
Operator: Admin User

Approved: 2/15/2021 2:22:29 PM
Beads Per Pellet: 47100
Cytometer SN: Z6591800015
Director:
Department: None

Entry Status: Approved
Software: BD FACSuite Clinical v1.4
Institution: None
Address:

Tube Name: Stem Cell + 7-AAD

Events Acquired	89,332	Acquisition Date	7/28/2020
Stem Cell Reagent Lot ID	0111871	Acquisition Time	5:03:10 PM
7-AAD Lot ID	0111890	Acquisition Duration (sec)	180.0
Sample Type	Fresh Bone Marrow	Dilution Factor	1.0
Keyword 1	Test 1	Pack Volume (mL)	20.0
Keyword 2	Test 2	Body Weight of Recipient (kg)	50.0

Results Summary

Label	Events/Value	Abs Cnt (cells/µL)
Bead Events	4,057	
Viable CD45+	73,442	① 8,526
Viable CD34+ Stem Cell	447	② 52
Total CD45+		③ 9,155
Total CD34+ Stem Cell		④ 54
Viable CD34+ Stem Cell as % of Viable CD45+	⑤ 0.61	
Total CD34+ Stem Cell as % of Total CD45+	⑥ 0.59	
CD34+ Stem Cell Viability (%)	⑦ 96.34	
CD45+ Viability (%)	⑧ 93.13	
Viable CD34+ Stem Cell per Body Weight of Recipient (cells/kg)	⑨ 20,758	
Viable CD34+ Stem Cell per Pack (cells/pack)	⑩ 1,037,895	

QC Messages

• Viable CD45+ gate does not contain requested 75,000 events

Showing 1 of 1 QC Messages

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Stem Cell + 7-AAD v1.0
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Printed: 2/15/2021 2:22:29 PM

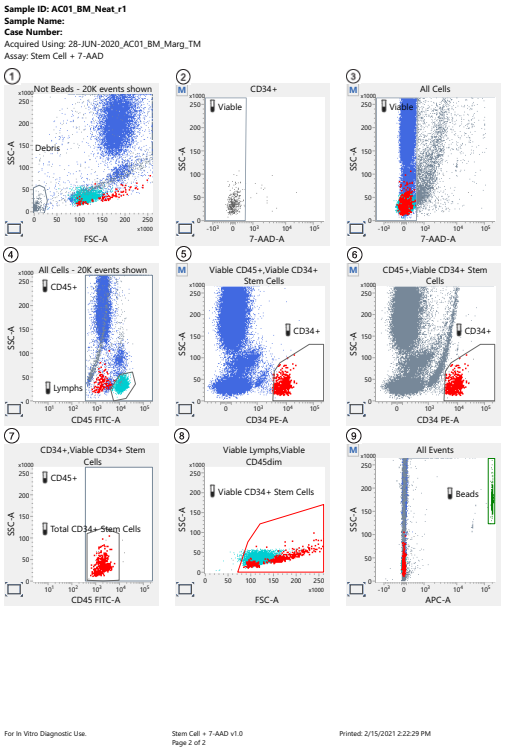
Result	Equation
Sample volume	(Bead events × specimen volume [µL])/BD Trucount™ bead count (on pouch label)
1	(Viable $CD45^+$ events/sample volume) × dilution factor
2	(Viable $CD34^+$ stem cell events/sample volume) × dilution factor

Result	Equation
3	(Total $CD45^+$ events[a]/sample volume) × dilution factor
4	(Total $CD34^+$ stem cell events[b]/sample volume) × dilution factor
5	(Viable $CD34^+$ stem cell absolute count/viable $CD45^+$ absolute count) × 100%
6	(Total $CD34^+$ stem cell absolute count/total $CD45^+$ absolute count) × 100%
7	(Viable $CD34^+$ stem cell absolute count/total $CD34^+$ stem cell absolute count) × 100%
8	(Viable $CD45^+$ absolute count/total $CD45^+$ absolute count) × 100%
9	(Viable $CD34^+$ stem cell per pack/kg body weight of recipient) = cells/kg
10	(Viable $CD34^+$ absolute count × pack volume × 1,000 µL/mL) = cells/pack

a. To obtain this value, hover over the CD45+ gate in the SSC-A vs CD45 FITC-A dot plot (plot 4). The number of events is in the tooltip.

b. To obtain this value, hover over the Total CD34+ Stem Cells gate in the SSC-A vs CD45 FITC-A dot plot (plot 7). The number of events is in the tooltip.

3. Inspect the dot plots on page 2 of the lab report and adjust the gates, as needed. Enlarge each dot plot to more easily gate the relevant cell population.



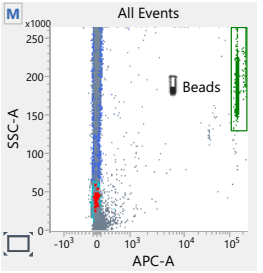
Plot	Gate(s)	Plot	Gate(s)
1	Debris	6	CD34+
2	Viable	7	CD45+, Total CD34+ Stem Cells
3	Viable	8	Viable CD34+ Stem Cells
4	CD45+, Lymphs	9	Beads
5	CD34+		

Gating strategy

This section explains the gating strategy used to analyze the sample. All of the gates, with the exception of the Debris gate, are autogates and, in most cases, will not need to be adjusted. However, we recommend that you review each dot plot. The example shown is for a fresh peripheral blood specimen.

Analyze the sample using the following gating strategy:

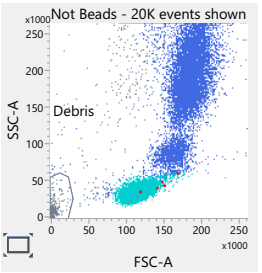
1. Identify BD Trucount™ beads in the APC-A vs SSC-A dot plot (plot 9).



The dot plot displays all ungated events. Events in the Beads gate appear green.

Action: Extend the Beads gate off the right edge of the dot plot to include all beads events, if needed.

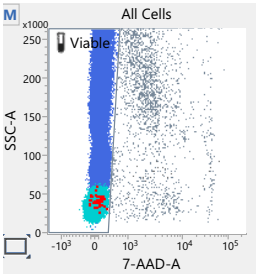
2. Exclude debris in the FSC-A vs SSC-A dot plot (plot 1).



The dot plot displays all events that are not beads. Events in the Debris gate appear gray.

Action: Adjust the gate to encompass the debris in the lower-left corner of the dot plot. The remaining ungated events are the All Cells population.

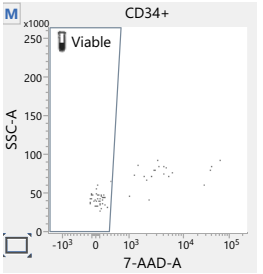
3. Identify the viable cells (7-AAD–negative events) in the 7-AAD-A vs SSC-A dot plot from the All Cells population (plot 3).



The plot displays all events excluding beads and debris. Events in the Viable gate include $CD45^+$ (blue), lymphocyte (light blue), and $CD34^+$ (red) populations.

Action: Confirm that the Viable gate encompasses only $7\text{-}AAD^-$ events.

4. Confirm the viable cells in the 7-AAD-A vs SSC-A dot plot from the CD34+ population (plot 2).

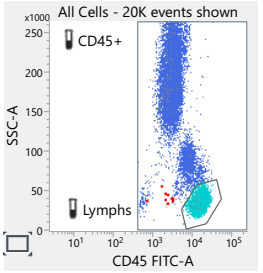


Viable and non-viable CD34+ events, identified in plots 5 and 6, are displayed.

Action: Confirm that the Viable gate encompasses only 7-AAD⁻ events.

Note: This is the only place that non-viable CD34+ events are displayed (7-AAD+).

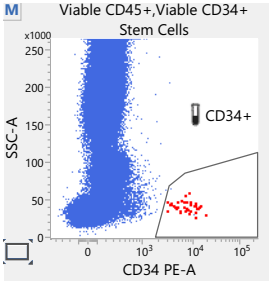
5. Identify the lymphocytes in the CD45 FITC-A vs SSC-A dot plot from the All Cells population (plot 4).



The plot displays all events excluding beads and debris. CD45+ cells (blue) and lymphocytes (light blue) are identified using the CD45+ gate and the Lymphs gate, respectively.

Actions: Adjust the CD45+ gate to include all events, including all CD45^{dim} cells. Adjust the Lymphs gate to encompass the lymphocyte population.

6. Identify the CD34+ events among the viable CD45+ events in the CD34 PE-A vs SSC-A dot plot (plot 5).

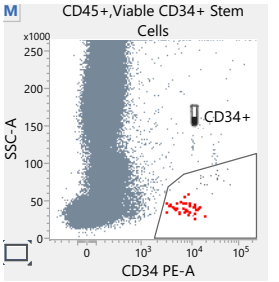


Viable CD45+ events from plots 3 and 4 are displayed. CD34+ events (red) are identified using the CD34+ gate.

Action: Adjust the CD34+ gate to encompass the cluster of cells in the lower right of the plot. Do not reduce the height of the gate.

Note: Non-viable CD34+ events are not displayed.

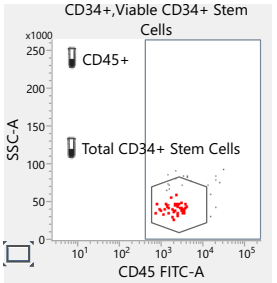
7. Exclude non-viable CD45+ events from the CD34+ population in the CD34 PE-A vs SSC-A dot plot (plot 6).



Total CD45+ events (viable and non-viable) from plot 4 are displayed. This gate is used to remove platelet streaks and other debris from the analysis.

Action: Adjust the CD34+ gate to exclude any CD45+ events that are visible in this plot, but not in plot 5.

8. Identify the Total CD34+ Stem Cell events in the CD45+ FITC-A vs SSC-A dot plot (plot 7).

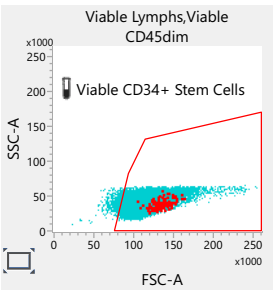


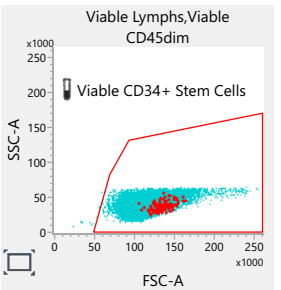
All CD45+ events from the CD34+ population, identified in plots 5 and 6, are displayed.

Action: Confirm that the Total CD34+ Stem Cells gate encompasses the cluster of CD45dim cells within the CD45+ gate.

Note: The height of the gate is the same as in plots 5 and 6. The gate is set by the algorithm on total CD34+ cells, which is necessary for CD34 viability estimation.

9. Identify stem cells as SSC^{low} events in the FSC-A vs SSC-A dot plot (plot 8). The example shown is a fresh leukapheresis sample.





Viable lymphocytes, identified in plots 3 and 4, and viable CD45dim events, identified in plot 7 using the Total CD34+ Stem Cells gate, are displayed.

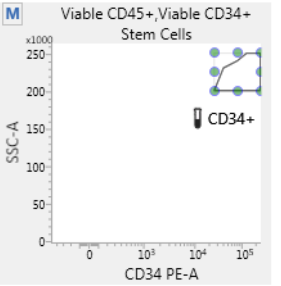
Action: Adjust the Viable CD34+ Stem Cells gate to encompass the viable lymphocytes (light blue).

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:

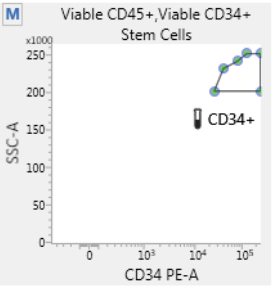
1. Click on a gate in the dot plot so that the gate is in bound mode.



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.

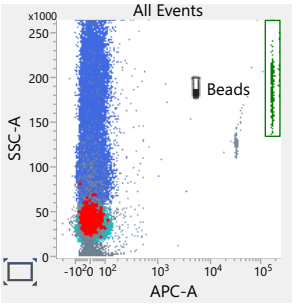
   See the *BD FACSLyric™ Clinical Reference System* for more information.

Gating example: mobilized peripheral blood specimen

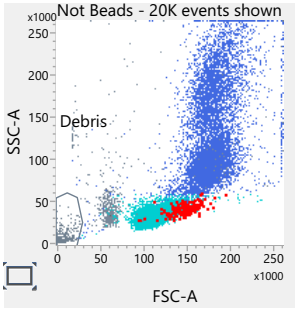
This section shows the gating strategy for a mobilized peripheral blood specimen. None of the gates in the example were manually adjusted.

Review the dot plots in the lab report in the following order.

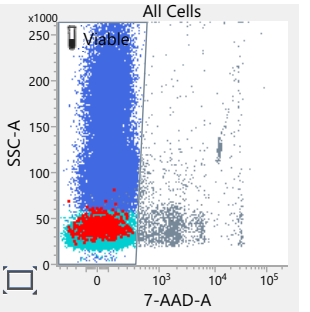
1. Plot 9:



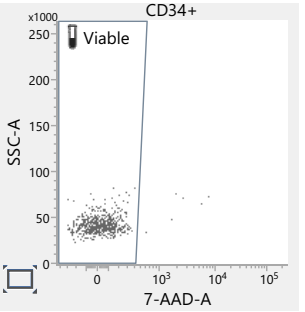
2. Plot 1:



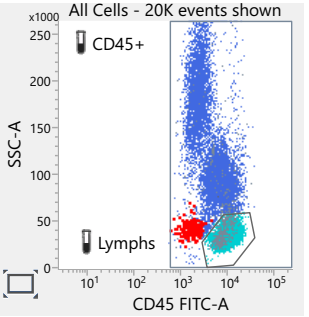
3. Plot 3:



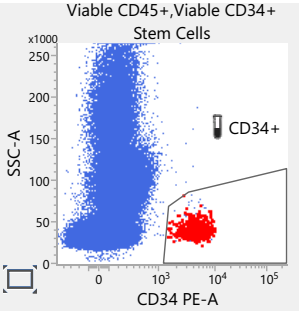
4. Plot 2:



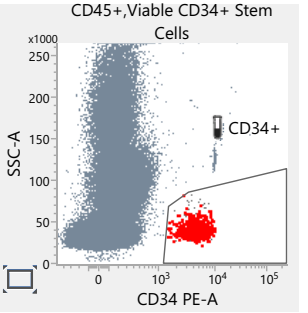
5. Plot 4:



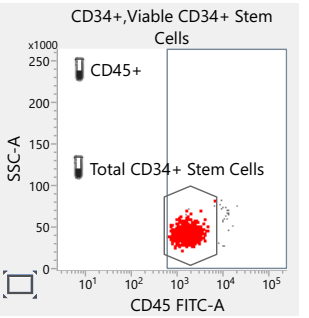
6. Plot 5:



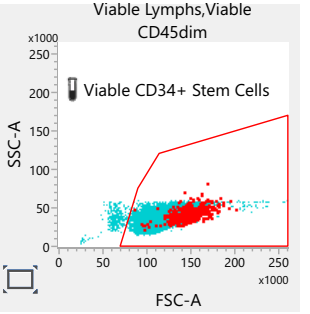
7. Plot 6:



8. Plot 7:



9. Plot 8:

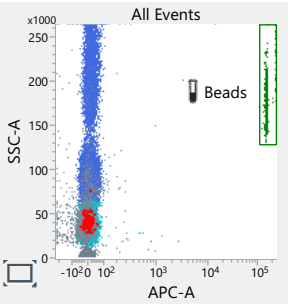


Gating example: leukapheresis specimen (fresh)

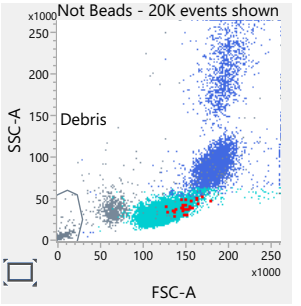
This section shows the gating strategy for a fresh leukapheresis specimen. None of the gates in the example were manually adjusted.

Review the dot plots in the lab report in the following order.

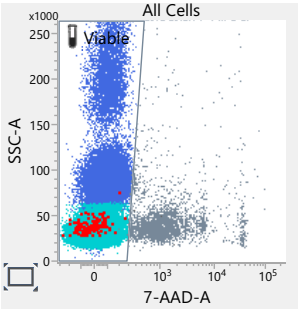
1. Plot 9:



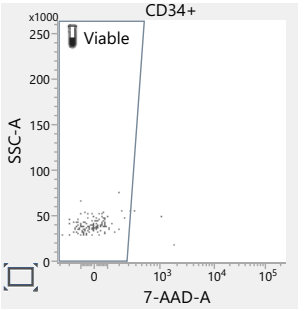
2. Plot 1:



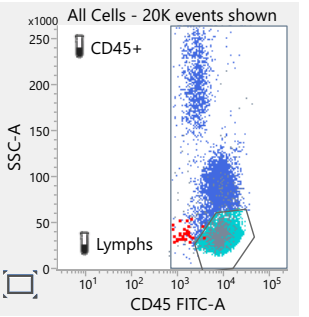
3. Plot 3:



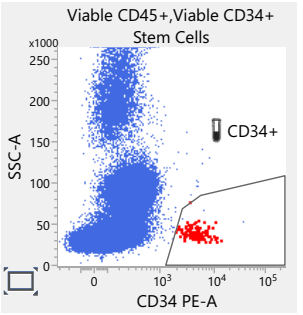
4. Plot 2:



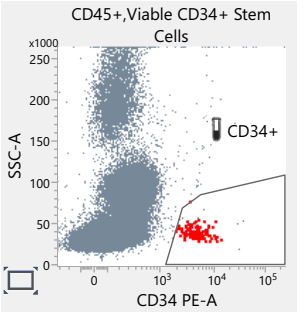
5. Plot 4:



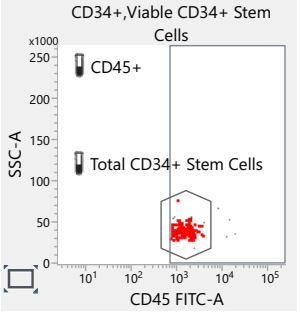
6. Plot 5:



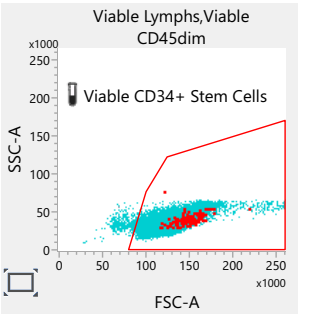
7. Plot 6:



8. Plot 7:



9. Plot 8:

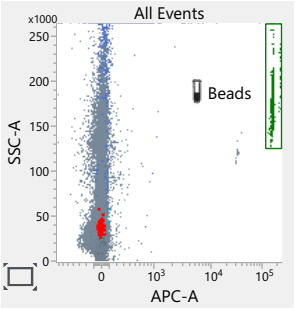


Gating example: leukapheresis specimen (post-thaw)

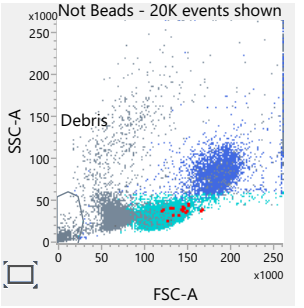
This section shows the gating strategy for a leukapheresis specimen post-thaw. None of the gates in the example were manually adjusted.

Review the dot plots in the lab report in the following order.

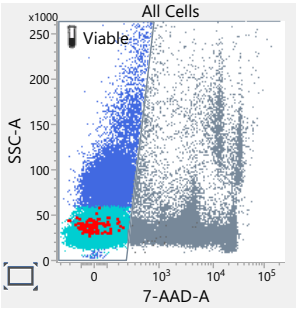
1. Plot 9:



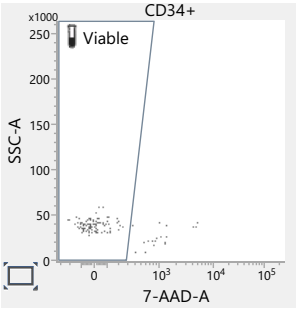
2. Plot 1:



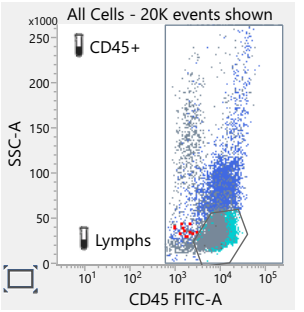
3. Plot 3:



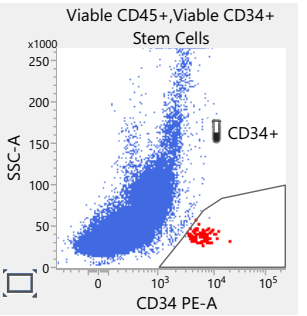
4. Plot 2:



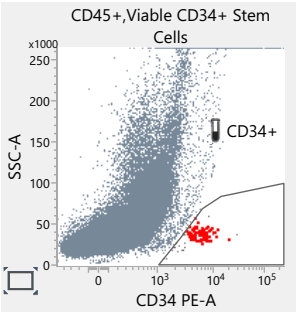
5. Plot 4:



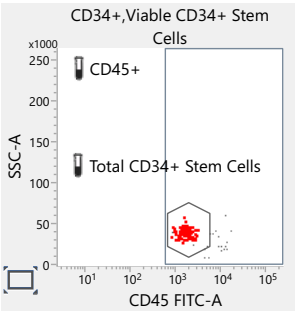
6. Plot 5:



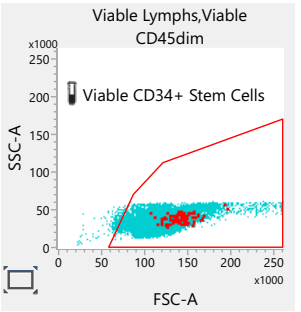
7. Plot 6:



8. Plot 7:



9. Plot 8:

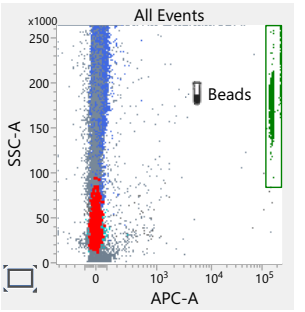


Gating example: bone marrow specimen (fresh)

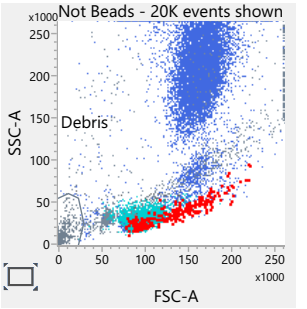
This section shows the gating strategy for a fresh bone marrow specimen. The CD34+ gate in plot 6 was manually adjusted to exclude the platelet streak.

Review the dot plots in the lab report in the following order.

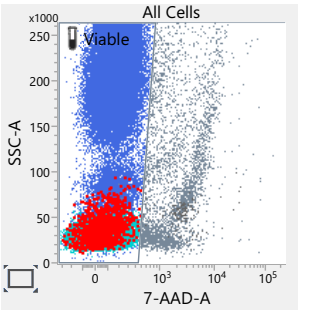
1. Plot 9:



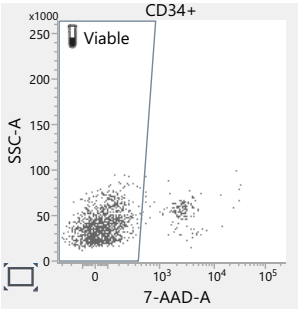
2. Plot 1:



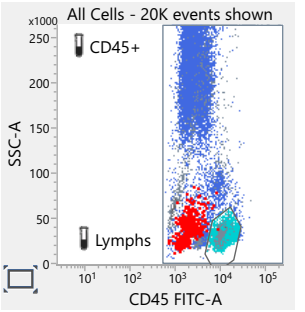
3. Plot 3:



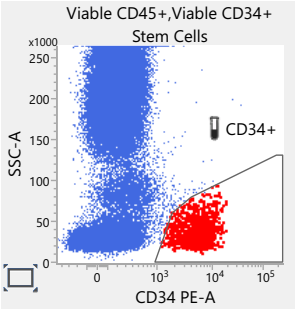
4. Plot 2:



5. Plot 4:

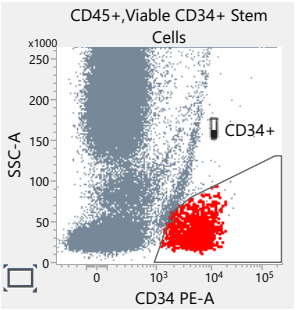


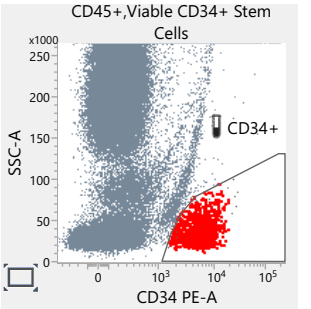
6. Plot 5:



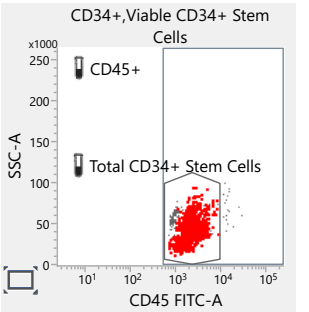
7. Plot 6:

   The gate in the bottom panel was adjusted to remove the gray population representing the platelet streak.

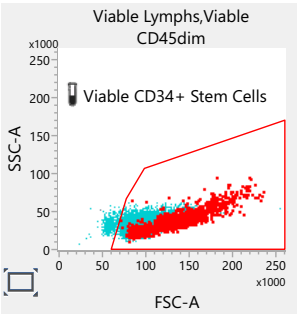




8. Plot 7:



9. Plot 8:

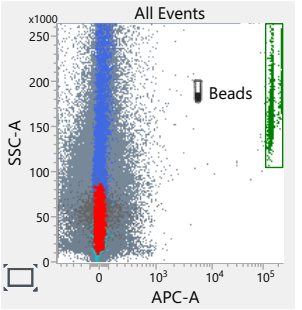


Gating example: bone marrow specimen (post-thaw)

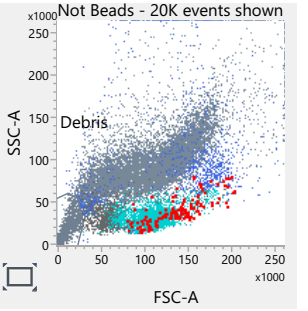
This section shows the gating strategy for a bone marrow specimen post-thaw. None of the gates in the example were manually adjusted.

Review the dot plots in the lab report in the following order.

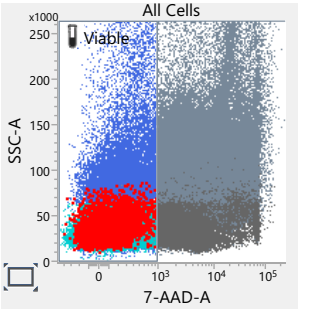
1. Plot 9:



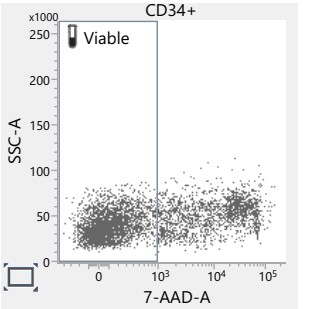
2. Plot 1:



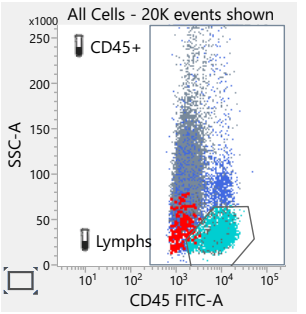
3. Plot 3:



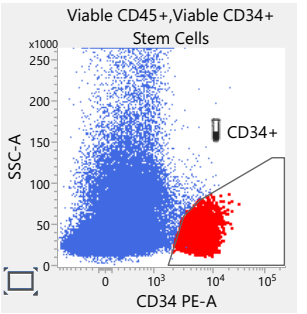
4. Plot 2:



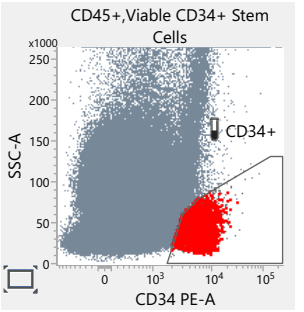
5. Plot 4:



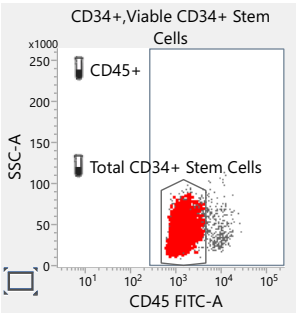
6. Plot 5:



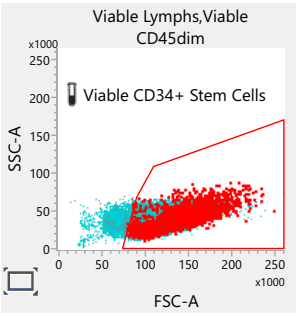
7. Plot 6:



8. Plot 7:



9. Plot 8:

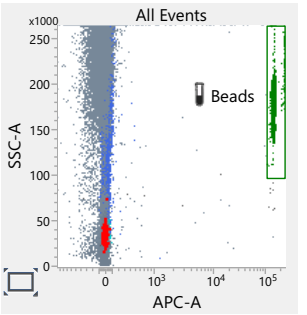


Gating example: cord blood specimen (fresh)

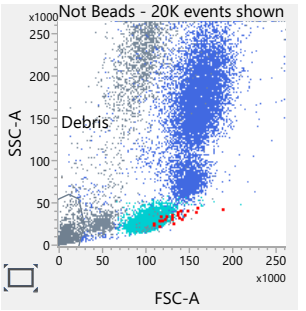
This section shows the gating strategy for a fresh cord blood specimen. None of the gates in the example were manually adjusted.

Review the dot plots in the lab report in the following order.

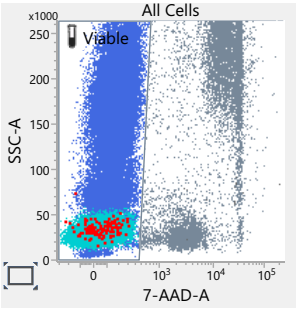
1. Plot 9:



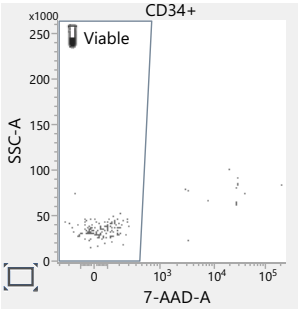
2. Plot 1:



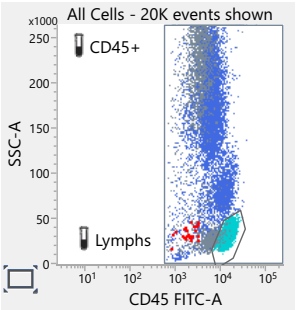
3. Plot 3:



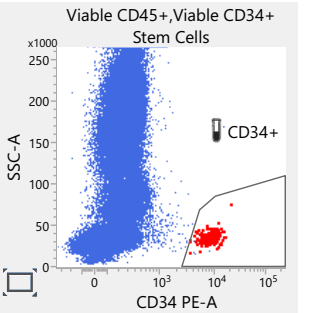
4. Plot 2:



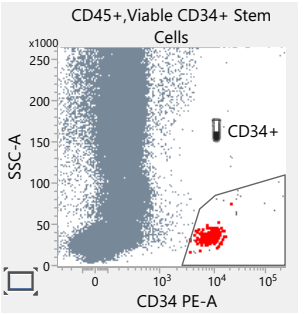
5. Plot 4:



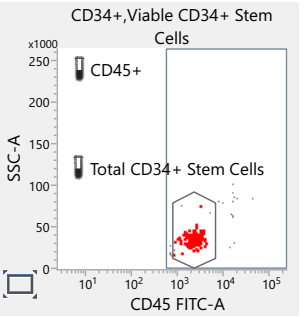
6. Plot 5:



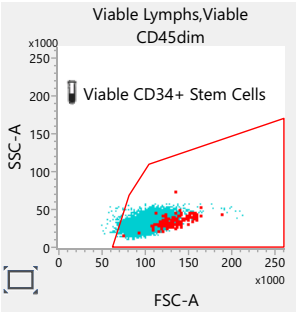
7. Plot 6:



8. Plot 7:



9. Plot 8:

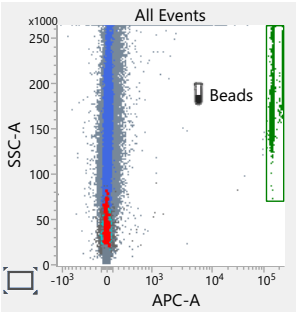


Gating example: cord blood specimen (post-thaw)

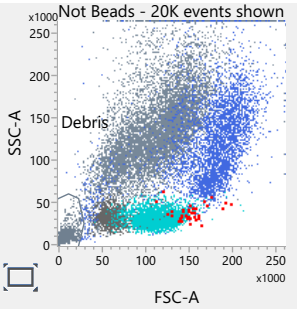
This section shows the gating strategy for a cord blood specimen post thaw. None of the gates in the example were manually adjusted.

Review the dot plots in the lab report in the following order.

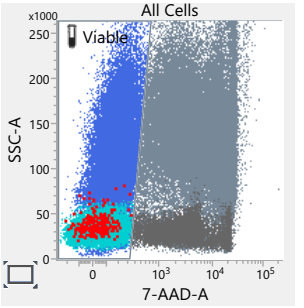
1. Plot 9:



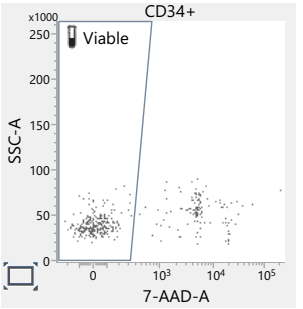
2. Plot 1:



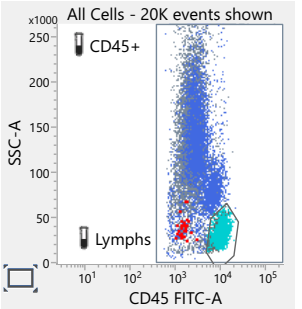
3. Plot 3:



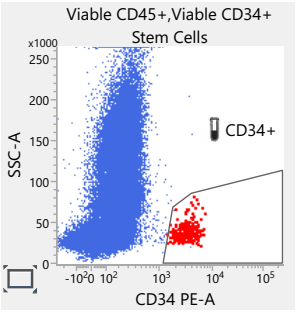
4. Plot 2:



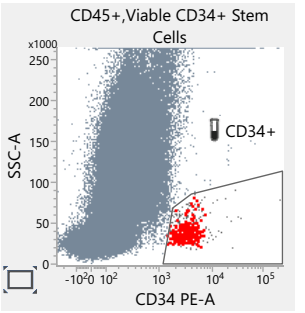
5. Plot 4:



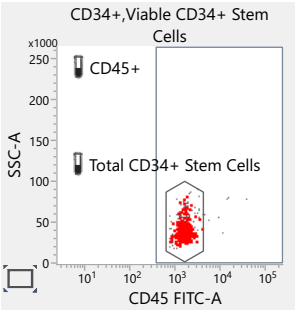
6. Plot 5:



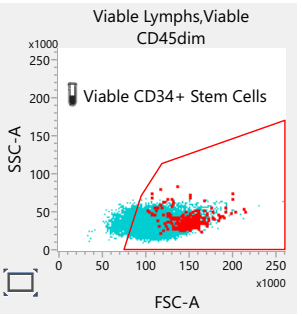
7. Plot 6:



8. Plot 7:



9. Plot 8:


Viable Lymphs,Viable CD45dim
Viable CD34+ Stem Cells
SSC-A
FSC-A
x1000
250
200
150
100
50
0
0
50
100
150
200
250
x1000

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3

Troubleshooting

This chapter covers the following topics:

- Troubleshooting overview (page 58)
- No results reported (page 59)
- Incomplete results reported (page 60)
- Gate location is suspect, but all results are reported (page 60)
- Insufficient number of events collected, but all results are reported (page 62)
- General warnings (page 63)
- Heparin anticoagulant (page 65)

Troubleshooting overview

This chapter lists problems you might encounter when using the BD® Stem Cell Enumeration 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® Stem Cell Enumeration 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 7) for information.

No results reported

QC message	Possible cause	Recommended solution
Viable Gate Failed. Place gates manually.	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 controls.	Repeat staining without adding 7-AAD to the process controls and acquire the newly stained process controls. See the *BD® Stem Cell Enumeration Kit* instructions for use.
$CD45^+$ Gate Failed. Place gates manually.	Insufficient number of $CD45^+$ cells in the sample.	Manually re-gate the sample. If manual re-gating does not remove the error, then repeat staining and acquire the newly stained sample.
Lymphs Gate Failed. Place gates manually.	Insufficient number of lymphocytes in the sample.	Manually re-gate the sample. If manual re-gating does not remove the error, then repeat staining and acquire the newly stained sample.
	Unresolved lymphocyte population.	Manually re-gate the sample. If manual re-gating does not remove the error, then repeat staining and acquire the newly stained sample.
$CD34^+$ Gate Failed. Place gates manually.	Insufficient number of $CD34^+$ cells in the sample.	Manually re-gate the sample. If manual re-gating does not remove the error, then repeat staining and acquire the newly stained sample.
Total $CD34^+$ Stem Cell Gate Failed. Place gates manually.	Insufficient number of total $CD34^+$ stem cells in the sample.	Manually re-gate the sample. If manual re-gating does not remove the error, then repeat staining and acquire the newly stained sample.
Viable $CD34^+$ Stem Cell Gate Failed. Place gates manually.	Insufficient number of viable $CD34^+$ stem cells in the sample.	Manually re-gate the sample. If manual re-gating does not remove the error, then repeat staining and acquire the newly stained sample.

Incomplete results reported

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.
Beads gate does not contain requested 1,000 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.

Gate location is suspect, but all results are reported

QC message	Possible cause	Recommended solution
Viable gate failed algorithm QC. Verify gate placement.	Unusual gate location.	Manually re-gate the sample.
$CD45^+$ gate failed algorithm QC. Verify gate placement.	Unusual gate location.	Manually re-gate the sample.
Lymphs gate failed algorithm QC. Verify gate placement.	Unusual gate location.	Manually re-gate the sample.

QC message	Possible cause	Recommended solution
$CD34^+$ gate failed algorithm QC. Verify gate placement.	Unusual gate location.	Manually re-gate the sample.
Total $CD34^+$ Stem Cell gate failed algorithm QC. Verify gate placement.	Unusual gate location.	Manually re-gate the sample.
Viable $CD34^+$ Stem Cell gate failed algorithm QC. Verify gate placement.	Unusual gate location.	Manually re-gate the 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.

Insufficient number of events collected, but all results are reported

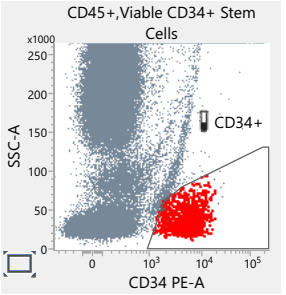
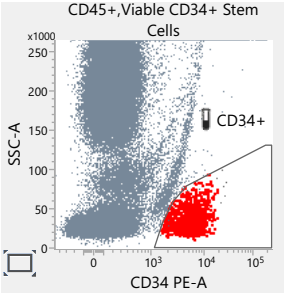
QC message	Possible cause	Recommend solution
Viable $CD45^+$ gate does not contain requested 75,000 events	Insufficient number of viable cells in the sample. Acquisition timed out after 10 minutes.	1. Review the plots and lab report. 2. If needed, stain and acquire a new specimen.
	The sample is too dilute.	Use a smaller dilution factor, repeat staining, and then acquire the newly stained sample.
Viable $CD34^+$ Stem Cells gate does not contain requested 125 events	Low $CD34^+$ count. Acquisition timed out after 10 minutes.	1. Review the plots and lab report. 2. If needed, stain and acquire a new specimen.
	Low $CD34^+$ count. Acquisition timed out before 10 minutes.	1. Review the plots and lab report. 2. If needed, stain and acquire a new specimen.
	Poor resolution between $CD34^+$ cells and other cells. Acquisition timed out before 10 minutes.	1. Review the plots and lab report. 2. If needed, stain and acquire a new specimen.

General warnings

QC message	Possible cause	Recommended solution
WBC count (cells/µL) value is X. Value must be ≤45,000.	WBC count is too high (>45,000 cells/µL).	1. Dilute the specimen. 2. Re-stain the specimen and acquire it.
Acquired without completed Assay Setup	Assay Setup was not completed.	1. Re-run Assay and Tube Settings setup and verify that setup is successful. 2. Re-run the sample.
Acquired with Assay Setup that passed with warnings.	7-AAD was not added to the tube.	Add 7-AAD to the 7-AAD–stained tube and re-run Assay and Tube Settings setup.
Acquired with failed Assay Setup.	7-AAD was not added to the tube.	Add 7-AAD to the 7-AAD–stained tube and re-run Assay and Tube Settings setup.
Acquired with expired Assay Setup.	Assay Setup using 7-AAD was not run.	1. Re-run Assay and 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.

QC message	Possible cause	Recommended solution
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.
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: BD Stem Cell Enumeration Kit.	The BD® Stem Cell Enumeration 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™ Tube has expired.	Re-stain sample in a BD Trucount™ Tube that has not expired, and acquire the newly stained sample.
Reference Settings were created with expired FC beads.	Reference settings were updated using expired BD® FC Beads.	1. Update reference settings using BD® FC Beads that have not expired. 2. Re-run the sample or stain a new specimen and acquire it.
Acquired with expired reference settings.	Reference settings have expired.	1. Update reference settings. 2. Re-run the sample or stain a new specimen and acquire it.
Acquired without defining spillover values.	Reference settings were not created.	Create reference settings using BD® FC Beads.

Heparin anticoagulant

Observation	Possible cause	Recommended solution
Platelet streak.	A sample was collected in heparin anticoagulant.	1. In plot 6, adjust the left boundary of the CD34+ gate to exclude the platelets.  2. After adjusting the gate, the CD34+ population should not include gray events representing the platelet streak. 

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