BD Stem Cell Enumeration Application Guide

for BD FACSCalibur Flow Cytometers

IVD For In Vitro Diagnostic Use CE

bdbiosciences.com
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Class 1 Laser Product.

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History

Revision	Date	Change Made
640784 Rev. A	3/2008	Initial release
23-11206-00 Rev. A	11/2009	Updated to add comments from European clinical trials (performance evaluation studies).
23-11206-01 Rev. 01	12/2011	Updated to add Class 1 laser information and heading for intended use.

Contents

Conventions

The following tables list conventions in this guide. Table 1 lists the symbols that are used in this application guide or on safety labels to alert you to a potential hazard. Text and keypad conventions are shown in Table 2.

Table 1 Hazard symbols[1]

Symbol	Meaning
	**CAUTION** hazard or unsafe practice that could result in material damage, data loss, minor or severe injury, or death

1. This symbol appears on the instrument with the mandatory yellow background per requirement of the applicable standard.

Table 2 Text and keypad conventions

Convention	Use
☑**Tip**	highlights features or hints that can save time and prevent difficulties
NOTE	describes important features or instructions
Italics	highlight book titles and new or unfamiliar terms on their first appearance in the text.
[ ]	identify screen keys, for example, [Run]
MM/DD/YY	indicates the date, in month, day, year; eg, 08/09/06
HH:MM:SS	indicates time, in hours, minutes, seconds; eg, 13:23:48

Technical Assistance

For technical questions or assistance in solving a problem:

- See Troubleshooting on page 30.
- Read the section of the *BD FACSCalibur Instructions for Use*, or the *BD CellQuest* or *BD CellQuest Pro Software Reference Manual* specific to the operation you are performing.

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, have the following information available:

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

Introduction

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 This Guide

This guide provides instructions for acquiring and analyzing samples stained using the BD Stem Cell Enumeration kit on a BD FACSCalibur flow cytometer. The *BD Stem Cell Enumeration* CD included with this application guide contains acquisition and analysis templates created in BD CellQuest software v3.3 (OS 9) and BD CellQuest Pro software v4.0.2, v5.2.1, and v6.0 (OS 9, OS X, Intel-based Mac® Pro computer). The templates are based on the method featured in the Clinical and Laboratory Standards Institute H42-A2 approved guideline* and include plots, regions, gates, statistics, and equations to determine the percentage of positive and the absolute number of $CD34^+$ cells in stained samples. Instructions for both BD CellQuest and BD CellQuest Pro software are provided. See the *BD Stem Cell Enumeration Kit* package insert for staining instructions.

* *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.

Running Instrument Setup

See the appropriate instrument, software, and reagent instructions for use for directions for setting up the BD FACSCalibur instrument.

Setting Up the Instrument

1 Start the BD FACSCalibur instrument and then turn on the computer.

2 Set up the cytometer using BD Calibrite™ beads and BD FACSComp™ software with 3-color or 4-color lyse/no-wash (LNW) settings. Verify that all parameters pass.

NOTE You can use either 3-color or 4-color LNW settings to set up the cytometer. The 4-color settings do not interfere with the assay.

See the *BD Calibrite Beads* package insert and the *BD FACSComp Software Reference Manual* for staining and setup instructions.

3 Quit BD FACSComp software.

4 Copy either the BD CellQuest Pro template or the BD CellQuest template from the *BD Stem Cell Enumeration* CD to your hard drive.

See page 26 and page 28 for examples of each template.

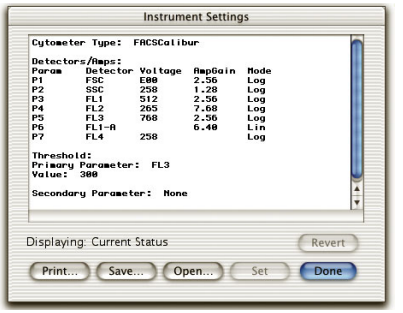
5 Launch BD CellQuest or BD CellQuest Pro software.

6 Select File > Open > BD SCE CQ Template 1.0 RevA or BD SCE CQ Pro Template 1.0 RevA.

7 Select Acquire > Connect to Cytometer.

If instrument settings have not been changed since the last BD FACSComp software run, go to Optimizing and Adjusting Compensation, page 9.

8 Select Cytometer > Instrument Settings.



- Click Open in the Instrument Settings window.
- Select BD Files > Instrument Settings Files. Click Open and navigate to *Calib File.LNW*. Double-click the file icon.
- Click Set, and then click Done.

Optimizing and Adjusting Compensation

1 Prepare the High process control, press RUN, put the control on the sample injection probe (SIP), and then press HI on the cytometer.

See the *BD Stem Cell Control* package insert for instructions on preparing the control.

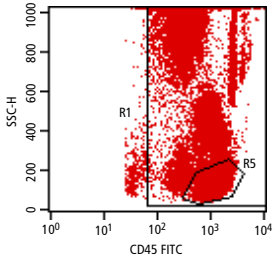
2 In the Acquisition Control window, select the Setup checkbox, and then click Acquire to begin instrument optimization.

3 Select Cytometer > Threshold.

- In the Threshold window, change FL3 to FL1.
- Monitor Plot 1 (FL1 vs SSC) while adjusting the FL1 threshold as necessary, to exclude debris without excluding any CD45 cells. See Figure 1 on page 10.

☑**Tip** Set the FL1 threshold to 300 and adjust as necessary.

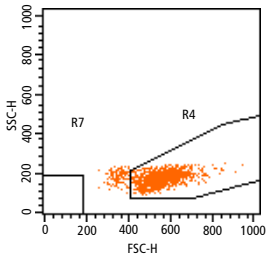
Figure 1 Plot 1 (CD45 vs SSC)



4 Select Cytometer > Detectors/Amps.

Observe Plot 6 (FSC vs SSC dot plot, Figure 2) and, if necessary, adjust the FSC gain in the Detectors/Amps window, so that the viable lymphocytes fall between channels 400 and 600.

Figure 2 Plot 6 (FSC vs SSC)



R7 is an acquisition exclusion gate used to exclude debris. R7 should not exceed FSC channel 200 and SSC channel 200 and should not encroach the lymphocyte population, or cells in this region will be excluded from the data file.

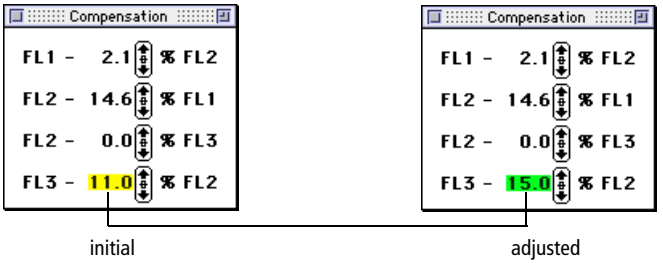
5 Select Cytometer > Compensation.

- In the Compensation window, note the initial value for FL3–%FL2, and increase this value by 4 (Figure 3 on page 11).

⚠ Failure to increase compensation can result in inaccurate data and an underestimation of viable $CD34^+$ cells.

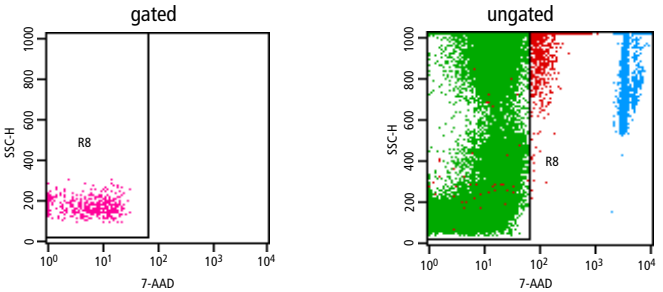
See Troubleshooting for help with compensation.

Figure 3 Compensation window, initial and adjusted



- Observe the two 7-AAD v SSC plots (Figure 4). One is ungated and one is gated on the CD34 Total gate. Both plots display R8. Because this sample does not have 7-AAD in the tube, all CD34 cells fall in the negative gate (R8).

Figure 4 7-AAD vs SSC dot plots, gated and ungated



- On the ungated plot, adjust R8 to include all the 7-AAD$^-$ cells.
- On the gated plot, verify that the CD34 cells are all within R8. Adjust FL3–%FL2 compensation if any CD34$^+$ cells are outside the gate.

6 Observe the remaining plots and adjust according to the description in Gating Strategy on page 22.

7 In the Acquisition control window, select Pause, then Abort. Clear the Setup checkbox.

8 Remove the High control from the SIP.

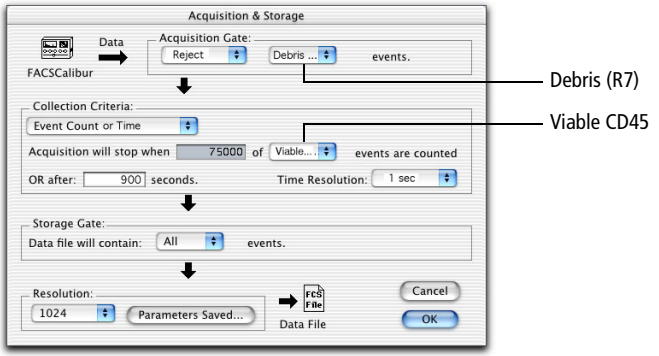
Setting Acquisition and Storage Criteria

Samples that complete acquisition in less than 15 minutes must meet the following conditions to be reported:

- At least 100 viable CD34 events
- At least 1,000 beads
- At least 75,000 viable CD45 events

1 To set acquisition and storage criteria, in the Parameter Description or Browser window, specify a file name and storage location for the data files.

2 Verify the following options in the Acquisition & Storage window.

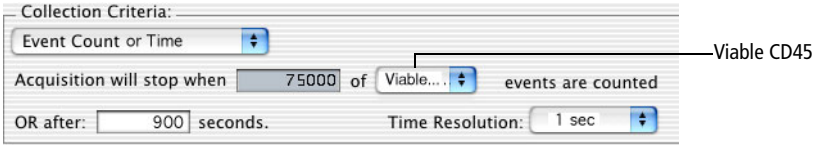


- Confirm the acquisition gate. Select to Reject Debris (R7) events.

- Confirm the Collection Criteria. Select *Event Count or Time*. Enter 75,000 in the *Acquisition will stop when* field, and set the menu to

Viable CD45 events are counted. Enter 900 seconds in the *OR after* field.

NOTE If the required number of events is not collected in 900 seconds (15 minutes), the software will stop acquisition and save the file.



Using Custom Keywords

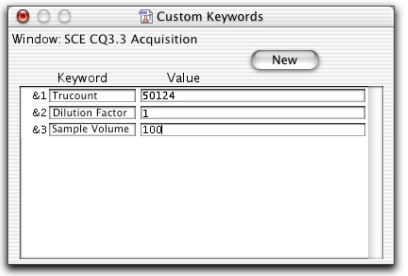
We recommend using the Custom Keywords option in both BD CellQuest and BD CellQuest Pro software to keep track of the BD Trucount™ bead count, the dilution factor, and the sample volume.

NOTE Information entered in these fields will be saved in the data file as long as these entries are made before the data file is acquired.

1 Open the BD Stem Cell Enumeration acquisition template.

2 Select Acquire > Custom Keywords.

NOTE The cytometer must be connected.



3 Enter the correct bead count in the Trucount Value field.

4 If the specimen was diluted, enter the dilution factor in the Dilution Factor Value field.

NOTE The Sample Volume value should be 100 at all times for this assay.

5 Close the Custom Keywords window.

NOTE Do not click the New button in the window.

The BD Trucount bead count will be shown in the Gate Stats view header if you are working in OS 9. In OS X, the bead count will be shown after the document has been saved and then reopened.

☑**Tip** These values will be exported with the statistics for future calculations in your spreadsheet, but only if they are displayed in the Stats view.

Acquiring and Analyzing Process Controls

See the *BD Stem Cell Enumeration Kit* package insert for instructions on staining process controls.

Acquiring Controls

⚠ Due to the temperature requirements of this assay, the BD FACS™ Loader cannot be used to acquire samples.

1 Gently vortex the control and place it on the SIP.

2 Click Acquire.

- For both the High and Low BD Stem Cell controls, acquire and save 75,000 CD45 events each.
- For the BD Stem Cell control tube stained to test the 7-AAD reagent performance, acquire approximately 10,000 total events, click Pause,

and then click Save. (See the 7-AAD Reagent Performance section in the package insert.)

Analyzing Controls

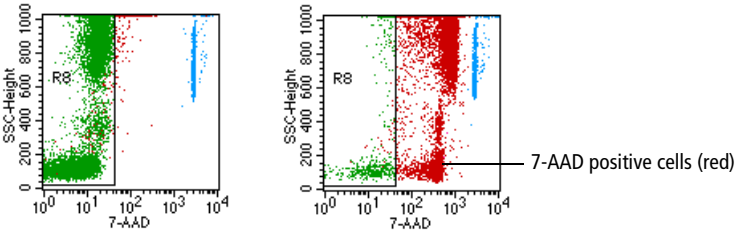
1 Read the data file for the first control into the template.

2 For both the High and Low BD Stem Cell controls, move the right border of R8 against the lymphocytes, monocytes, and granulocytes.

3 Check each plot and verify that the gates enclose the appropriate populations.

4 Verify that results are within the established range for the process controls you are using.

5 For the BD Stem Cell control stained with the 7-AAD reagent, verify that most of the cells appear outside Region R8.



no 7-AAD with 7-AAD

Acquiring, Reviewing, and Analyzing Samples

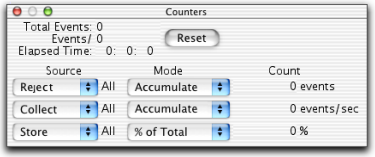
See the *BD Stem Cell Enumeration* kit package insert for instructions on staining cells.

Acquiring Samples

☑**Tip** For each sample, review the results and verify that the acquisition criteria have been met before acquiring the next sample, especially if sample volume is limited.

⚠ Due to the temperature requirements of this assay, the BD FACS™ Loader cannot be used to acquire samples.

1 Display the Counters window to monitor the acquired events.



2 Gently vortex the sample and place it on the SIP.

3 On the cytometer, press RUN and then HI.

4 In the software, click Acquire.

☑**Tip** After acquisition, save the Experiment document so that it is available for analysis.

Reviewing Results

1 Verify that the following conditions have been met.

It is important that you verify that **all** of the following conditions have been met for samples that complete acquisition in less than 15 minutes.

- At least 100 viable CD34 events
- At least 1,000 beads
- At least 75,000 viable CD45 events

2 If less than 100 viable CD34 events were acquired, but the other acquisition criteria were met, do the following:

3 Change Acquisition and Storage settings to acquire 100 events in the viable CD34 gate.

4 Continue to run the sample until 100 viable CD34 events are acquired.

5 Change the stopping criteria back to 75,000 viable CD45 events before continuing to the next sample.

6 Repeat the steps in Acquiring Samples on page 16 and Reviewing Results on page 17 for the remaining samples.

Analyzing Samples

Use this procedure if you are analyzing data with either BD CellQuest or BD CellQuest Pro software.

1 Read the data file for the first sample into the template.

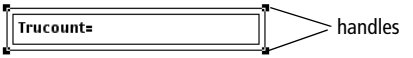
2 Check each plot and verify that the gates enclose the appropriate populations.

3 Follow the appropriate steps based on the software you are using (page 18 for BD CellQuest Pro software or page 19 for BD CellQuest software).

Analyzing Using BD CellQuest Pro Software

Do not delete regions from the plots by selecting them and then pressing the Backspace key. This will delete the regions from the document. The gates will become inactive and the Expression Editor calculations will disappear. If this does occur, reload the template.

1 Select the Trucount= text box by clicking the border (handles appear).

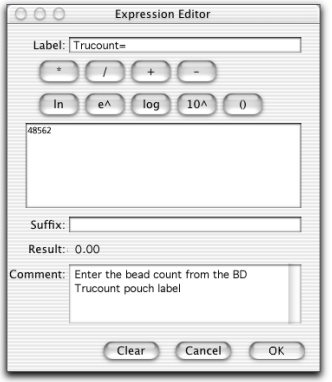


2 From the menu bar, select Statistics.

3 From the menu, select Edit Expression.

Alternatively, click the Expression Editor button  in the tool palette.

4 In the Expression Editor, enter the numbers for the bead count (beads/pellet).



5 To change the dilution factor, follow steps 1 through 4 for the dilution factor text box.

NOTE The default value for no dilution is 1.

⚠ If the numbers for the sample dilution factor or BD Trucount beads/pellet are not entered exactly, results will be incorrect.

6 View the calculated results.

7 Print the analysis for your records.

Analyzing Using BD CellQuest Software

If you are using BD CellQuest software with the BD CellQuest template, you must calculate results manually or export the statistics and calculate them using a spreadsheet validated by your laboratory. Follow this procedure to calculate statistics.

1 Enter the appropriate values from the Gate statistics into each of the following equations.

Label	Expression
Viable CD34 cells/µL =	$\frac{\text{Viable CD34 x BD Trucount x Dilution Factor}}{\text{Beads x Sample Volume}}$
Viable CD45 cells/µL =	$\frac{\text{Viable CD45 x BD Trucount x Dilution Factor}}{\text{Beads x Sample Volume}}$
Total CD34 cells/µL =	$\frac{\text{Total CD34 x BD Trucount x Dilution Factor}}{\text{Beads x Sample Volume}}$
CD34 % Viability =	$\frac{\text{Viable CD34 x 100}}{\text{Total CD34}}$
Viable CD34 % of Viable CD45 =	$\frac{\text{Viable CD34 x 100}}{\text{Viable CD45}}$

NOTE The values in the equations represent the gate names in the Gate Statistics view.

2 Enter the bead count from the BD Trucount pouch label.

3 Enter the dilution factor.

4 Enter the sample volume (100 µL).

If the numbers for the BD Trucount beads/pellet or the dilution factor are not entered exactly, results will be incorrect.

Consider the following example:

The absolute number of viable CD34 cells in a sample with a dilution factor of 1, a bead count of 53,514 beads per tube, and the Gate statistics shown in Figure 7 on page 27 would be:

$$\text{Viable CD34 cells/µL} = \frac{1262 \times 53514 \times 1}{6937 \times 100} = 97.35/\mu L$$

5 Repeat steps 1 through 4 for the remaining samples.

Gate Definitions

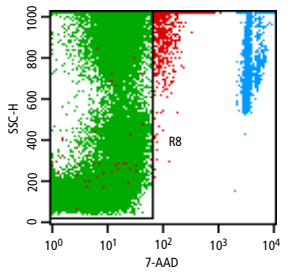
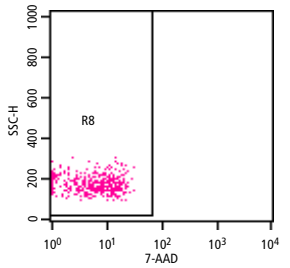
The plots and gating strategy for BD CellQuest and BD CellQuest Pro templates are the same. Results are automatically calculated by BD CellQuest Pro software, while BD CellQuest software requires manual calculation of results. Figure 5 shows gate definitions for BD CellQuest and BD CellQuest Pro software.

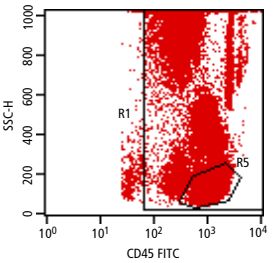
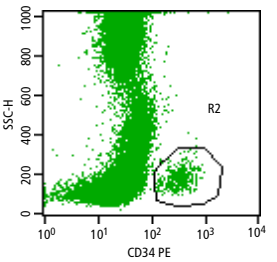
Figure 5 Gate definitions for BD CellQuest and BD CellQuest Pro software

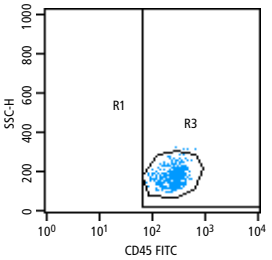
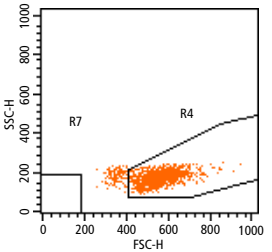
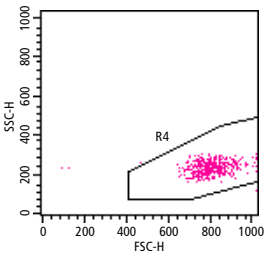
Color	Label	Definition
	Viable CD45	R1 and R8
	G2	R2 and "Viable CD45"
	G3	R3 and G2
	Viable CD34	R4 and G3
	Beads	R6
	Total CD34	R1 and R2 and R3
	Viable lymphs	R5 and R8
	Total CD45	R1 and not Beads
	Debris	R7

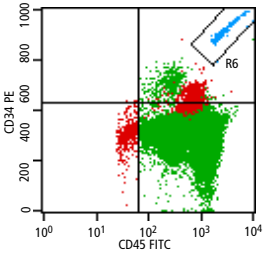
Gating Strategy

The following table explains the template gating strategy.

Dot Plot	Explanation
 **Plot 8: 7-AAD vs SSC**	This plot contains the first gate to be adjusted and is used to identify viable cells (7-AAD$^-$). Adjust R8 to enclose the 7-AAD$^-$ events. The region extends beyond the top of the plot on the y-axis, beyond the far left of the plot on the x-axis, and excludes positive-stained cells. ⚠ If there are a large number of red (dead) events outside R8, see page 39 for troubleshooting information.
 **Plot 7: 7-AAD vs SSC**	This plot is gated on the Total CD34 gate (R1 and R2 and R3) and is used during compensation optimization (FL3%–FL2). See page 11. This plot displays total CD34 cells and confirms proper adjustment of region R8 on Plot 8. If the instrument is appropriately set up and compensated, the gated viable CD34 cells will exhibit the same level of fluorescence as the viable lymphocytes in Plot 8.

Dot Plot	Explanation
 **Plot 1: CD45 vs SSC**	This plot is ungated and is used to include all CD45^{dim} to CD45^{bright} events and excludes debris, platelets, and unlysed red blood cells (RBCs), which are all CD45^{-}. Adjust the R1 leucocyte gate to extend above the top of the plot to include high SSC events that are CD45^{+}. Adjust the left side of the gate to include all CD45^{+} cells, including dim CD45^{+} events that are CD34^{+}. The right side can extend to the edge of the plot. Adjust polygon region R5 around the lymphocytes. Include only as many events as necessary to define the viable lymphocyte population displayed in Plot 6. ☑**Tip** Display fewer events on this plot so that the limits of the lymphocytes are easier to define.
 **Plot 2: CD34 vs SSC**	This plot displays viable CD45 (G1) cells and is used to identify CD34^{+} cells. Adjust R2 to include all CD34^{+} events. **NOTE** This region should exclude any platelets that form a streak between neutrophils and CD34^{+} events. See page 38 to view a plot showing a platelet streak.

Dot Plot	Explanation
 **Plot 3: CD45 vs SSC**	This plot displays G2 cells and is used to further define the viable CD34+CD45+ cell population. Adjust R3 to include only those events that form a cluster with low to intermediate SSC and $CD45^{dim}$ expression. Adjust R3 to exclude any lymphocytes or monocytes seen to the right of the stem cell cluster.
 **Plot 6: FSC vs SSC**	This plot displays Viable Lymphs (R5 and R8) and is used to establish the minimum FSC and SSC ranges for R4. Display region R4 on this plot and adjust the position so that only viable lymphs from R5 are included. R7 is an acquisition exclusion gate used to exclude debris. Region 7 should not exceed FSC channel 200 and SSC channel 200 and should not encroach the lymphocyte population because events in R7 will be excluded from the data file.
 **Plot 4: FSC vs SSC**	This plot displays G3 (R3 and G2) cells and is used to identify viable stem cells (CD34+). Adjust R4 to include only those events that form a cluster with low to intermediate SSC and medium to high FSC. The gate serves to exclude platelets and debris that can show weak, nonspecific binding of CD34 and CD45. Its lower FSC boundary is adjusted in Plot 6. Region R4 is the lymph/blast region and will be adjusted by scatter of lymphocytes. ☑**Tip** This plot can be gated on R1 and R2 and R3 to display Total CD34+ cells instead of Viable CD34+ cells.

Dot Plot	Explanation
 **Plot 5: CD45 FITC vs CD34 PE**	This ungated multicolor plot is used to identify beads (R6). Optional: Adjust the quadrant marker to establish a visual lower limit of CD45 expression by the $CD34^+$ events, as in Plot 1. The bead population appears in the top right corner. Allow R6 to extend beyond the plot boundary (x- and y-axes) to include all bead events. Verify that there are no cells in the bead gate.

Template Examples

Figure 6 and Figure 8 show templates without data. Figure 7 shows example data for BD CellQuest software. Figure 9 shows example data for BD CellQuest Pro software.

Figure 6 BD CellQuest template without data

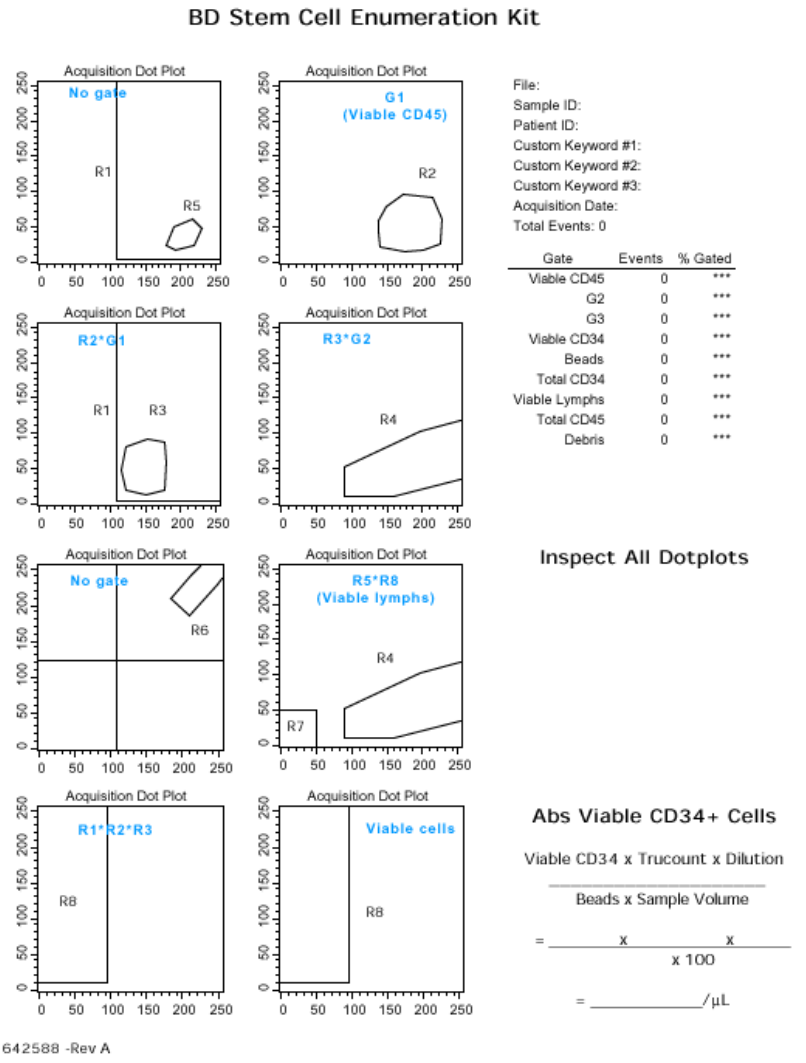


Figure 7 BD CellQuest template with example data

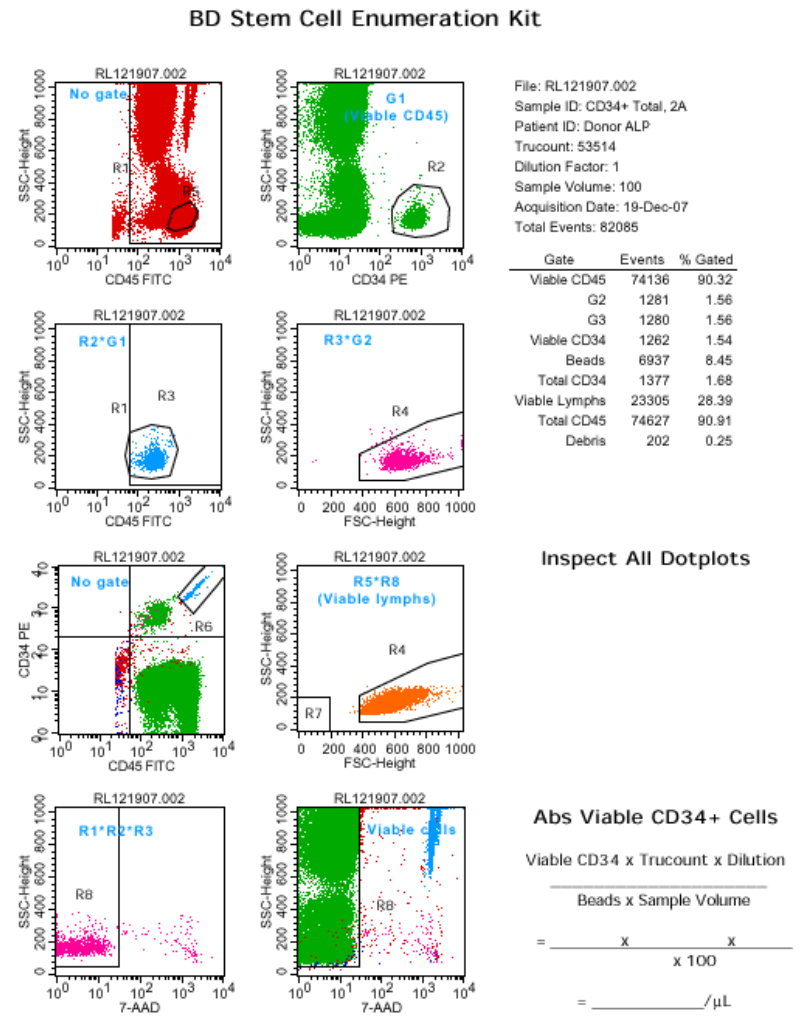


Figure 8 BD CellQuest Pro template without data

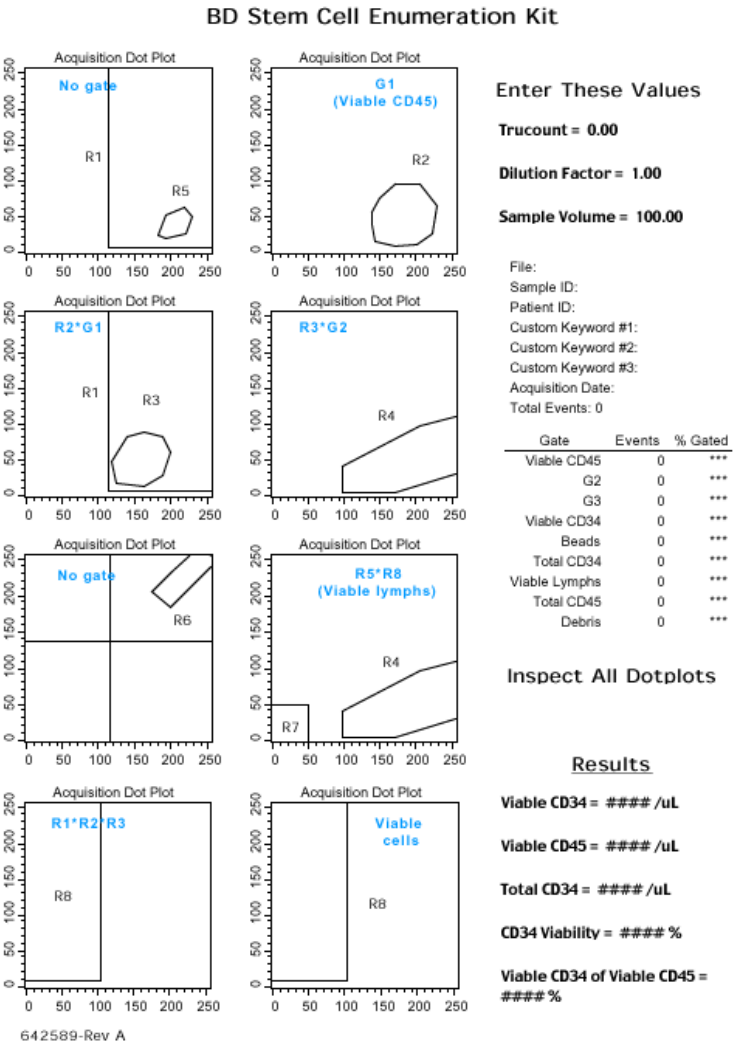
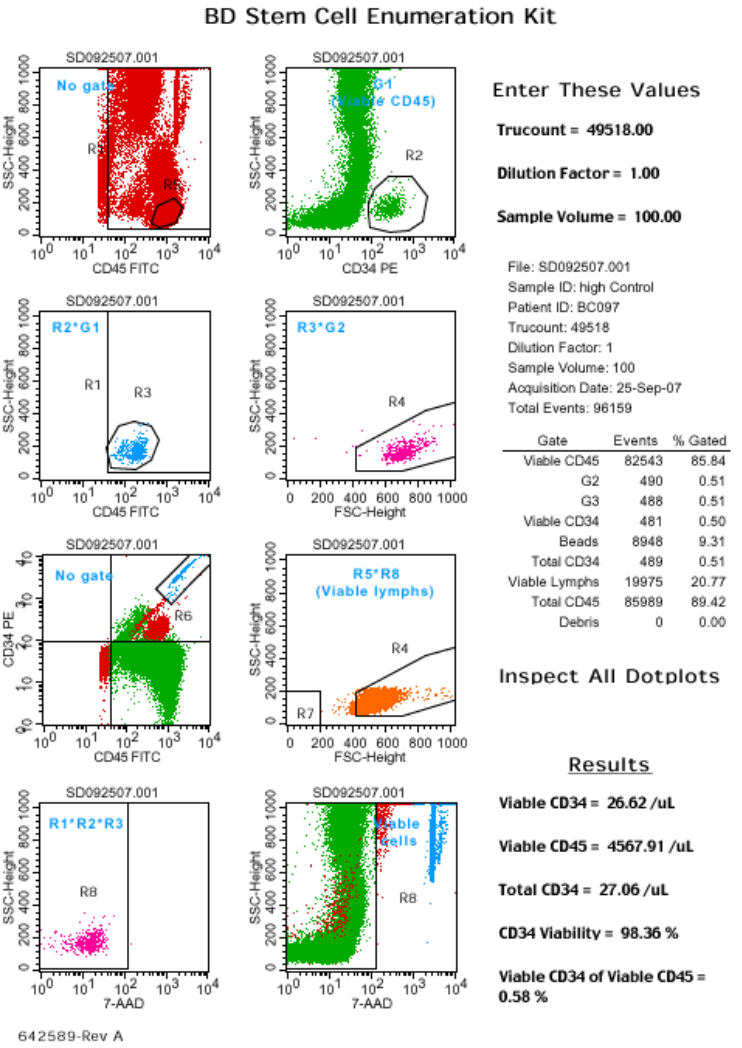


Figure 9 BD CellQuest Pro template with example data



Enter These Values

Trucount = 49518.00

Dilution Factor = 1.00

Sample Volume = 100.00

File: SD092507.001
Sample ID: high Control
Patient ID: BC097
Trucount: 49518
Dilution Factor: 1
Sample Volume: 100
Acquisition Date: 25-Sep-07
Total Events: 96159

Gate	Events	% Gated
Viable CD45	82543	85.84
G2	490	0.51
G3	488	0.51
Viable CD34	481	0.50
Beads	8948	9.31
Total CD34	489	0.51
Viable Lymphs	19975	20.77
Total CD45	85989	89.42
Debris	0	0.00

Inspect All Dotplots

Results

Viable CD34 = 26.62 /uL

Viable CD45 = 4567.91 /uL

Total CD34 = 27.06 /uL

CD34 Viability = 98.36 %

Viable CD34 of Viable CD45 = 0.58 %

Troubleshooting

The following section provides assistance for problems you might have using the BD Stem Cell Enumeration assay. If additional assistance is required, contact your local BD Biosciences technical support representative. See our website, bdbiosciences.com, for up-to-date contact information.

Troubleshooting

Observation	Possible Causes	Recommended Solutions
No events in acquisition display	No sample in tube	Add sample to the tube or install a new sample tube.
	Sample not mixed properly	Vortex the sample to suspend the cells and beads.
	Sample injection tube clogged or air bubble in the flow cell	See the cytometer manual for instrument instructions.
	Communication failure between computer and flow cytometer: GPIO error, cannot read instrument status	**1** Turn off the computer and the flow cytometer. **2** Check the GPIO cable connections. **3** Turn on the cytometer. Then, turn on the computer.

Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Status window displaying STNDBY	Flow cytometer not in RUN mode	Select the RUN mode.
	Sample tube not installed or not properly seated	Install the sample tube on the cytometer.
	Sample tube cracked	Vortex the tube and transfer the contents of the cracked tube to a 12 x 75-mm tube. Do **not** use another BD Trucount tube. ⚠ Proper mixing is required to ensure all beads are transferred to the new tube.

Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Status window displaying STNDBY (continued)	Sheath tank cap not tightened	Tighten the sheath tank cap.
	Sheath tank bracket not replaced	Install the bracket. See the cytometer manual.
	Worn seals	Replace the seals. See the cytometer manual.
	Cytometer in Loader configuration	Change the tube interface to manual. See the Loader manual for instructions. ⚠ Due to the temperature requirements of this assay, the BD FACS Loader cannot be used to acquire samples.
	Vent valve toggle switch in incorrect position (vented sheath tank)	Flip the toggle switch to the opposite direction.
	Sheath tank tubing or sheath filter tubing not properly connected	Check that all tubing connectors are securely seated. Check the sheath tank for cracks.
	Sheath tank bracket not depressing pressure button under bracket	Tighten the screw on the top right-hand side of the bracket.
Status window displaying NOT READY	Laser not functioning	Check the laser power in the status window. If the power is 0 mWatts, turn off the instrument and computer. Then turn on the instrument followed by the computer. If the power is still 0 mWatts, contact your BD Biosciences service representative.

Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Status window displaying NOT READY (continued)	Leak at sheath area	See the cytometer manual for instructions.
	Sheath tank empty or waste tank full	Check the tanks. Fill the sheath tank and empty the waste tank, if necessary. See the cytometer manual for instructions.
High sample rate: >5,000 events per second	Air bubble in flow cell	See the cytometer manual for instructions.
	Air in sheath filter	Vent air from the sheath filter. See the cytometer manual for instructions.
	Sample too concentrated	Dilute the sample. Check the WBC count on the original sample. Dilute if the count is too high (>40,000 WBCs/µL) and restain. See the *BD Stem Cell Enumeration Kit* package insert for instructions.
	Instrument settings adjustment necessary	Rerun BD FACSComp software using the 3-color or 4-color LNW setup. See Setting Up the Instrument on page 8, and Optimizing and Adjusting Compensation on page 9.
Low sample rate: <100 events per second	Air bubble in flow cell	Prime the system. See the cytometer manual for instructions.
	Sheath tank empty	Check the tanks. Fill the sheath tank and empty the waste tank, if necessary. See the cytometer manual for instructions.
	Sheath container leaking or not sealed properly	Check the sheath container and replace it if necessary. Check the cap gasket and replace it if necessary.

Troubleshooting (continued)

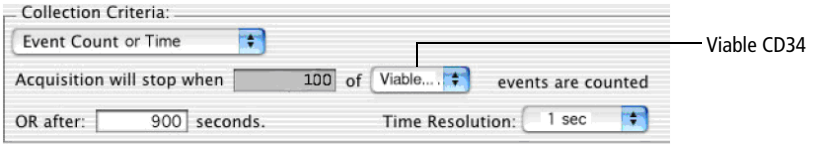
Observation	Possible Causes	Recommended Solutions
Low sample rate: <100 events per second (continued)	Sample tube cracked	Vortex the tube and transfer the contents of the cracked tube to a 12 x 75-mm tube. Do **not** use another BD Trucount tube.
	Sample not adequately mixed	Vortex the sample to suspend the cells and beads.
	Clog in sample injection tube	Call your BD Biosciences service representative.
	Flow rate not set to HI	Set the flow rate to HI.
Erratic event rate	Worn seals	See the cytometer manual for instructions.
	Cytometer in Loader configuration	Change the tube interface to manual. See the Loader manual for instructions. ⚠ Due to the temperature requirements of this assay, the BD FACS Loader cannot be used to acquire samples.
	Partially blocked sample injection tube	Call your BD Biosciences service representative.
	Sheath tank empty or waste tank full	Check the tanks. Fill the sheath tank and empty the waste tank, if necessary. See the cytometer manual for instructions.
Unusual population distribution	Air bubble in flow cell	See the cytometer manual for instructions.
	Air leak at sheath tank	See the cytometer manual for instructions.

Troubleshooting (continued)

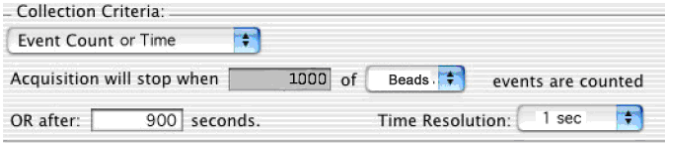
Observation	Possible Causes	Recommended Solutions
Unusual population distribution (continued)	Partially blocked sample injection port (SIP)	**1** Fill a tube with 3 mL of 10% CONTRAD® cleaning solution, and install on the SIP. **2** Move the support arm to the side and allow the vacuum to aspirate for 1 minute. **3** Move the support arm to the center and run the CONTRAD tube on HI for 10 minutes. **4** Remove the tube and Prime twice to remove CONTRAD from the SIP and flow cell. **5** Install a tube of DI water, run for 10 minutes, and set the cytometer to STNDBY when done.
	Sheath filter dirty or has air	Change the sheath filter or purge air. See the cytometer manual for instructions.
	Flow cell dirty	Perform the monthly cleaning procedure. See the cytometer manual for instructions.
	Improperly set seals on SIP tube	Replace the seals. See the cytometer manual.
	Cytometer in Loader configuration	Change the tube interface to manual. See the Loader manual for instructions. ⚠ Due to the temperature requirements of this assay, the BD FACS Loader cannot be used to acquire samples.
	Wrong parameters chosen in plot	Make sure that the plots follow the gating strategy. See page 22.

Troubleshooting (continued)

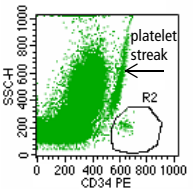
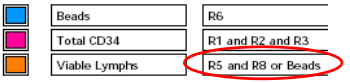
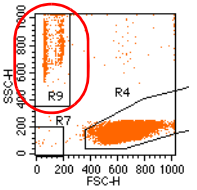
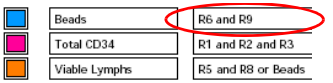
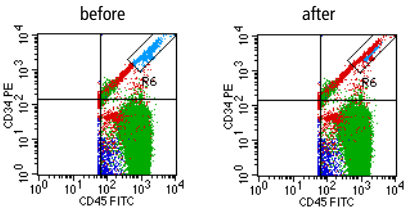
Observation	Possible Causes	Recommended Solutions
Unusual population distribution (continued)	Wrong threshold	Select FL1 at approximately 300. See step 3 on page 9.
	Instrument settings adjustment necessary	Follow the procedure Setting Up the Instrument on page 8.
Sample ran dry	Sample run more than once	Monitor the samples that are being re-run, since the software warnings cannot prevent re-run tubes from running dry.
	Sample in Setup mode too long before acquiring	Make sure that the sample is not in Setup mode for more than 1 minute before you acquire. Pause, clear the Setup checkbox, and then click Acquire.
	Sample aspirated to waste	Immediately center the tube support arm after installing the tube on the SIP.
Less than 100 viable CD34 cells acquired in 75,000 viable CD45 events	Specimen with <5 CD34 cells/µL	**1** Change Acquisition and Storage settings to acquire 100 events in the viable CD34 gate. See the figure. **2** Revert to the stopping criteria of 75,000 viable CD45 events before continuing to the next sample.
Less than 100 stem cells acquired in 15 minutes	Too few viable CD34 cells in the specimen	Accept the data. CD34 count is <5 CD34 cells/µL.



Troubleshooting (continued)

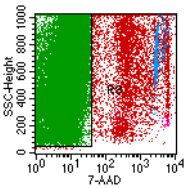
Observation	Possible Causes	Recommended Solutions
Less than 1,000 beads acquired in 75,000 viable CD45 events	Sample too concentrated	Select one of the following options: • Dilute the sample to <35,000 WBC/µL. • Change Acquisition and Storage settings to acquire 1,000 events in the Beads gate. See the figure. **NOTE** Revert to the stopping criteria of 75,000 viable CD45 events before continuing to the next sample. 
Less than 75,000 viable CD45 events	Specimen too dilute	If specimen was diluted, re-stain with undiluted specimen.
	Specimen with low viability	**1** Verify that 100 viable CD34 events and 1,000 beads were acquired. **2** Revert to the stopping criteria of 75,000 viable CD45 events before continuing to the next sample.
	Specimen with low WBCs	Acquire sample for 15 minutes.

Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Cells in bead gate (R6)	Platelet streak 	Reported values will be undercounted. Make these gating changes. See Figure 5 on page 21. **1** In the Gate List, add *or Beads* to the Viable Lymphs definition (R5 and R8), and press Enter.  **2** In Plot 6 (FSC vs SSC), create a new region (R9) to surround the bead population in the top left.  **3** In the Gate List add *and R9* to the Beads definition (R6), and press Enter.  

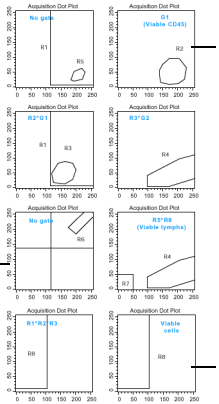
Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Many dead cells in Plot 8 causing viability to fluctuate with small changes to R2	Dead cells in R2 are being included in the Total CD34 count, causing lower than expected viability (see calculations on page 19)	Small changes to R2 cause large changes in the viability calculation or Total CD34 count. This is seen in older or thawed samples with many 7-AAD+ (non-viable) events. In the following example: • Viable cells are green • Dead CD34 cells are magenta • Other dead cells are red **1** While observing the Gate stats view, adjust R2 so that the Viable CD34 count is maximized while the Total CD34 count is minimized. **NOTE** Look for the CD34+ (magenta) cells on your computer screen. They should appear inside R2 and to the right of the viable population. ☑**Tip** If you prefer, ungate plot 2 to see where the dead (red) cells are interfering with the position of R2. Select Multicolor Gating in the Inspector. Be sure to set the gating back to viable CD45 before running the next sample.

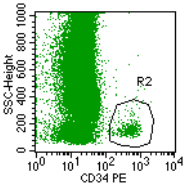


Plot 8

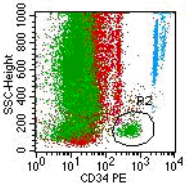
Position of plots on template



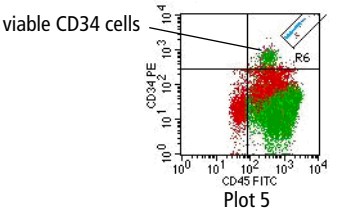
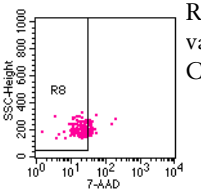
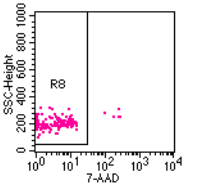
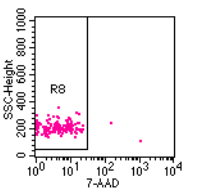
Plot 2 gated



Plot 2 ungated



Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Many dead cells in Plot 8 causing viability to fluctuate with small changes to R2 (continued)		**1** For quality control, observe Plot 5 (ungated quadrant plot). Verify that the CD34+ cells are above the horizontal quadrant and low in CD45.  Plot 5
Difficulty adjusting compensation	The sample is under compensated; only some of the CD34 cells fall inside R8	under compensated  Raise the FL3–%FL2 value to bring the CD34 cells inside R8.
	The sample is over compensated; some of the CD34 cells are off-scale	over compensated  Lower the FL3–%FL2 value to bring the CD34 cells off the left edge of the plot. Verify the left edge of R8 is off the plot to include all CD34 cells.
	The sample is properly compensated	proper compensation  The FL3 median of the total CD34 population is the same as the FL3 median of the lymphocyte population on R8. No adjustment needed.

Performance

For assay performance information, see the *BD Stem Cell Enumeration Kit* package insert.

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