



BD Procount™ Progenitor Cell Enumeration Kit

Reagent kit for enumerating CD34 cells by flow cytometry

25 Tests—Catalog No. 340498

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1. INTENDED USE

BD Procount™ progenitor cell enumeration kit is intended for use as an in vitro diagnostic test to identify and enumerate absolute counts and percentages of CD34+ cells in human peripheral blood samples, mobilized peripheral blood, and leukapheresis samples using flow cytometry.

2. SUMMARY AND EXPLANATION

Transplantation of hematopoietic progenitor cells is used increasingly in the treatment of blood disorders, malignancies, and genetic abnormalities.¹⁻³ Progenitor cells are rare and are found primarily in the bone marrow, with extremely low frequencies in peripheral blood. However, with the arrival of mobilization regimens (G-CSF, GM-CSF, and chemo-therapy), peripheral blood has become the preferred source of stem cells.¹⁻³

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.⁴

An accurate measure of the CD34 cell count is necessary for dose requirement protocols in stem cell transplantation.² An incorrectly high result could lead to an infusate with less than the recommended threshold dose of CD34+ cells. Quantitating the CD34+ cell population can also be useful during mobilization.

Fluorochrome-conjugated monoclonal antibodies directed against the CD34 molecule can be used to identify CD34+ cells by flow cytometry. Flow cytometric

applications for CD34⁺ cell identification and enumeration provide a rapid, quantitative, and reproducible method to evaluate the progenitor cell population.

Significant site-to-site variation has been observed with current flow cytometric methods to determine the percent and absolute numbers of CD34⁺ cells.⁵ The BD Procount assay incorporates BD Trucount™ tubes to volumetrically determine the absolute cell count, thereby eliminating any variability associated with hematology-derived absolute counts.

3. PRINCIPLES OF THE PROCEDURE

A two-tube assay is performed by staining the sample with appropriate reagents in individual BD Trucount tubes. When blood sample is added to the reagent, fluorochrome-labeled antibodies in the reagent bind specifically to cell-surface antigens, while the nucleic acid dye stains the DNA and RNA of all nucleated cells. The lyophilized pellet in the BD Trucount tube dissolves, releasing a known number of fluorescent beads.

The Control reagent is used to assess the amount of nonspecific antibody binding, particularly that caused by Fc receptors. BD FACS™ lysing solution is used to lyse erythrocytes before the sample is acquired on the flow cytometer.

During analysis, the absolute number of CD34⁺ cells in the sample can be determined by dividing the number of CD34 cellular events by the number of fluorescent bead events, then multiplying by the bead concentration.

4. REAGENTS

The BD Procount Progenitor Cell Enumeration Kit contains CD34 reagent, Control reagent, and BD Trucount tubes, sufficient for 25 tests. Each reagent is provided in phosphate-buffered saline (PBS) with bovine gelatin and 0.1% sodium azide.

- Vial A: The CD34 reagent contains a nucleic acid dye and PE-labeled murine monoclonal CD34, clone 8G12, and PerCP-labeled murine monoclonal CD45, clone 2D1 (HLe-1).⁶
- Vial B: The Control reagent contains a nucleic acid dye and PE-labeled murine monoclonal KLH γ_1 (IgG₁), clone X40, and PerCP-labeled murine monoclonal CD45, clone 2D1 (HLe-1).⁶
- BD Trucount tubes each contain a lyophilized pellet of 4.2- μ m fluorescent-dyed beads.

CD34 recognizes the human progenitor cell antigen (HPCA) present on immature hematopoietic precursor cells and all hematopoietic colony-forming cells in bone marrow and blood, including unipotent and pluripotent progenitor cells.⁴

Mouse γ_1 (IgG₁) recognizes a keyhole limpet hemocyanin (KLH) antigen not expressed on human cells.

CD45 recognizes a 180- to 220-kilodalton (kDa) human leucocyte antigen that is a member of the leucocyte common antigen (LCA) family.⁶ The CD45 antigen is present on all human leucocytes and is weakly expressed or not expressed on hematopoietic progenitor cells.

Mouse γ_1 (IgG₁), CD34, and CD45 antibodies are composed of mouse γ_1 heavy chains and kappa light chains.

The DNA/RNA-specific dye detects all nucleated cells including leucocytes and nucleated red blood cells.

Concentration values are listed in the following table:

CD34 Reagent	Concentration ($\mu\text{g/mL}$)
Nucleic acid dye	250
CD34 PE	2.5
CD45 PerCP	6.25

Control Reagent	Concentration ($\mu\text{g/mL}$)
Nucleic acid dye	250
IgG1 PE	2.5
CD45 PerCP	6.25

Precautions

- For in vitro diagnostic use. Not for use in therapeutic procedures.
- The addition of a precise volume of blood is critical. Pipettes must be calibrated to deliver exactly 50 μL of sample. Perform the reverse pipetting technique according to the pipette manufacturer's instructions.
- Bead count varies by lot of BD Trucount tubes. It is critical to use the bead count shown on the lot of BD Trucount tubes you are currently using when calculating the absolute CD34 count. Do not mix multiple lots of tubes in the same assay.
- Do not freeze the reagents or expose them to direct light during storage or incubation with cells. Keep the reagent vials dry.

- Do not use the reagents if you observe any change in appearance. Precipitation or discoloration indicates instability or deterioration.
- The antibody reagents contain sodium azide as a preservative; however, care should be taken to avoid microbial contamination, which could cause erroneous results.

WARNING The reagent solution contains a nucleic acid-binding dye. The toxicological properties of this dye have not been investigated. If inhaled or ingested, contact a physician immediately. If skin or eye contact occurs, wash with copious amounts of water.

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection^{7,8} and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

- BD FACS lysing solution is required and contains diethylene glycol and formaldehyde. Refer to the *BD FACS Lysing Solution* IFU for warnings.

Storage and Handling

It is important that you carefully follow these instructions. Store the reagent vials upright at 2°C–8°C. Use opened vials within 4 weeks. Expiration dating for closed vials is shown on the label.

Store BD Trucount tubes in their original foil pouch at 2°C–25°C. To avoid potential condensation, open the pouch only after it has reached room temperature and carefully reseal the

pouch immediately after removing a tube. Examine the desiccant each time you open the pouch. If the desiccant has turned from blue to lavender, discard the remaining tubes. Use tubes within 1 hour after removal from the foil pouch. Once the pouch has been opened, the tubes are stable for 1 month. Do not use beyond the expiration date indicated on the packaging.

5. INSTRUMENT

The BD Procount kit is designed for use on a flow cytometer equipped with appropriate computer hardware and software. The cytometer must be equipped with a 488-nm laser and must be capable of detecting light scatter (forward and side or orthogonal) and three-color fluorescence at the following wavelengths.

- 515–545 nm (FL1)
- 562–607 nm (FL2)
- >650 nm (FL3 or FL4, depending upon the instrument manufacturer)

The instrument must be able to threshold or discriminate on the FL1 parameter.

We recommend using a BD FACST[™] brand flow cytometer, such as the BD FACSCalibur[™], BD FACScan[™], or BD FACSort[™] flow cytometer, equipped with BD Procount software, version 2.0 or later, for automated data acquisition and analysis. Use BD Calibrite[™] 3 beads and BD FACSCComp[™] software, version 2.0 or later, to set photomultiplier tube (PMT) voltages, fluorescence compensation, and to check instrument sensitivity. Choose the lyse/no-wash option at the BD FACSCComp Set Up view to select the appropriate target values before running BD FACSCComp software.

For other flow cytometers, set up the cytometer for three-color acquisition following the manufacturer's recommendations. Setup should include setting or checking PMT voltages, fluorescence compensation, and instrument sensitivity.

NOTE Performance has not been established on stream-in-air cytometer systems.

6. SPECIMEN COLLECTION AND PREPARATION

Collect peripheral blood aseptically by venipuncture⁹ into a sterile BD Vacutainer[™] EDTA blood collection tube. Each test requires 100 µL of sample (50 µL per tube). Follow the collection tube manufacturer's guidelines for the minimum volume of blood to be collected. Collect leukapheresis samples according to manufacturer's instructions. Store anti-coagulated blood at room temperature (20°C–25°C) until ready for staining. Stain peripheral blood samples within 24 hours of collection. Stain other samples within 6 hours of collection.

Perform a white blood cell (WBC) count on all samples to be evaluated. If the WBC count is greater than 50.0×10^3 WBC/µL, dilute the sample with 1X PBS (calcium and magnesium free) with 2% fetal calf serum (FCS) and 0.1% sodium azide. Record the dilution factor for the calculation of the final absolute CD34, CD45, or nucleated cell count.

To prepare concentrated peripheral blood for a high control sample (see Quality Control on page 6), collect the mononuclear cells from 10 mL of normal peripheral blood over a Ficoll-Paque[™] gradient. After processing, resuspend the cells in 0.5 mL PBS with 2% FCS.

Interfering Conditions

Do not use previously fixed and stored patient specimens. Samples refrigerated before staining could give aberrant results. Hemolyzed or clotted samples should be rejected.

7. PROCEDURE

Reagent Provided

- BD Procount CD34 reagent (nucleic acid dye/CD34 PE/CD45 PerCP)
- BD Procount Control reagent (nucleic acid dye/ γ_1 PE/CD45 PerCP)
- BD Trucount tubes (25 tubes/pouch)

Reagents and Materials Required but Not Provided

- BD Calibrite™ 3 beads (Catalog No. 340486)
 - BD FACS lysing solution (10X), 100 mL (Catalog No. 349202)
- Refer to the *BD FACS Lysing Solution* IFU for dilution instructions and warnings.
- Reagent-grade (distilled or deionized) water
 - BD Vacutainer EDTA blood collection tubes or equivalent.
 - Vortex mixer
 - Micropipettor with tips
 - Bulk dispenser or pipettor (450 μ L) for dispensing 1X BD FACS lysing solution
 - 1X PBS (Dulbecco's modified, pH 7.2 $\pm$ 0.2; 0.01 mol/L PO_4 ; and 0.15 mol/L NaCl) with 2% FCS and 0.1% sodium azide (only if sample dilution is necessary)

- Sheath fluid, BD FACSFlow™ solution (Catalog No. 342003) or equivalent.

CAUTION Use only BD FACSFlow sheath fluid diluent to dilute BD Calibrite beads.

- Low control: normal peripheral blood, or a commercially available low control sample
- High control: umbilical cord blood sample, leukapheresis sample, a peripheral blood sample concentrated using Ficoll-Paque, or a commercially available high control sample.

Staining the Cells

Stain peripheral blood samples within 24 hours of collection. Stain all other samples within 6 hours of collection. Follow the manufacturer's recommendations for staining commercial controls. Lyse red blood cells following staining using diluted (1X) BD FACS lysing solution. Use care to protect the tubes from direct light. Perform the procedure at room temperature (20°C–25°C).

1. For each donor sample and the daily system controls, label two BD Trucount tubes *CD34* and *Control*.

Also label each tube with the sample identification number.

NOTE Before use, verify that the bead pellet is intact and within the metal retainer at the bottom of each BD Trucount tube. If this is not the case, discard the tube and replace it with another.

2. Pipette 20 μ L of CD34 reagent into the tube labeled *CD34* and 20 μ L of Control reagent into the tube labeled *Control*.

Pipette the reagent onto the side of the each tube just above the stainless steel retainer. Do not touch the bead pellet.

3. Pipette 50 μ L of well-mixed sample into each tube using a calibrated pipette.

Use a BD electronic pipette or use the reverse pipetting technique. Pipette the sample onto the side of the tube just above the retainer. Use a new tip for each tube.

NOTE Avoid smearing blood down the side of the tube. If blood remains on the side of the tube, it will not be stained with the reagent.

4. Cap tubes and vortex gently to mix. Incubate for 15 minutes in the dark at room temperature.
5. Add 450 μ L 1X BD FACS lysing solution to each tube.
6. Cap tubes and vortex gently. Incubate for 30 minutes in the dark at room temperature.

The cells are now ready to be analyzed on the flow cytometer. Protect stained samples from direct light. Vortex each tube before acquiring. If stained samples are to be run on the BD FACST[™] loader, vortex each tube immediately before placing into the loader carousel.

If samples are not acquired immediately after preparation, store peripheral blood or leukapheresis samples in the dark at 2°C–8°C for up to 24 hours. Acquire other samples within 6 hours after preparation. Vortex thoroughly just before acquisition.

Quality Control

A. Daily System Controls

Run a low control and a high control sample daily to optimize instrument settings and as a quality control check of the system. Use normal peripheral blood or a commercially available low control sample as a low control, and run one of the following as a high control: an umbilical cord blood sample, a leukapheresis sample, a commercially available high control sample, or a peripheral blood sample concentrated using Ficoll-Paque. (See Specimen Collection and Preparation on page 4.) Validate daily system controls in your laboratory.

Begin acquisition optimization with the daily system controls. Visually inspect the dot plots for the low and high controls. The CD34⁺ cell cluster should appear as shown in Figure 1 and Figure 2.

Figure 1 Daily low control—CD34 tube showing few events in CD34 cluster (R3 = CD34⁺ cells, R5 = beads)

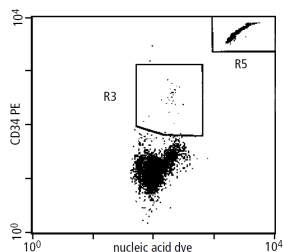
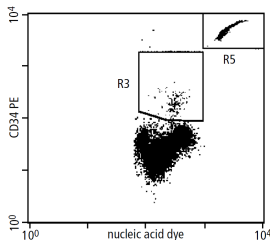


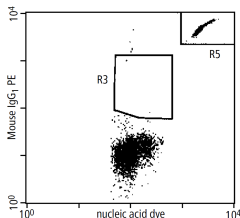
Figure 2 Daily high control—CD34 tube showing distinct CD34 cluster (R3 = CD34+ cells, R5 = beads)



B. Sample Controls

Run the tube containing the Control-stained sample after the tube containing the CD34-stained sample to assess nonspecific staining (Figure 3).

Figure 3 Control-stained sample showing very few events in CD34 region (R3 = CD34+ cells, R5 = beads)



NOTE Never subtract the Control-tube result from the CD34-tube result to try to correct for non-specific staining. Instead, adjust the region in BD CellQuest™ (or equivalent) software to reduce non-specific staining to within the QC criteria.

Before correcting for any dilution of sample, determine the absolute counts in the CD34 gate of the Control tube. If a Control tube meets one of the following criteria, it passes the nonspecific staining criteria.

- ≤ 10 events in the CD34 gate
- ≤ 4 cells/ μL in the CD34 gate
- CD34 cells/ $\mu\text{L} \leq 5\%$ of the CD34 tube

Using BD Procount Software on a BD Platform

Follow the instructions in the *BD Procount Software User's Guide* for data acquisition, automated analysis, and calculation of results.

Generic Flow Cytometry Instructions

BD Procount reagents are designed for use on a flow cytometer equipped with appropriate computer hardware and software. See Section 5, Instrument, on page 3 for specifications.

A. Instrument Setup

Follow the manufacturer's recommendations for setting up the cytometer for three-color acquisition. Setup should include setting or checking photomultiplier tube (PMT) voltages, fluorescence compensation, and instrument sensitivity. Once the instrument has been set up, use BD Calibrite 3 beads to adjust the compensation. Ensure the blank beads are in the lower left hand quadrant of the two-dimensional fluorescence displays.

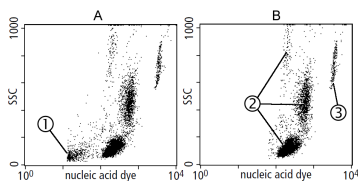
B. Sample Optimization

1. Use the acquisition software to create the following two-dimensional data displays.
 - nucleic acid dye vs SSC
 - CD45 PerCP vs SSC
 - nucleic acid dye vs CD34 PE
 - FSC (lin) vs SSC (lin) (used for optimization only)

- To begin optimization, set the FL1 threshold level to 250.
Different instruments might use different logarithmic and linear scales.
- Gently vortex the tube containing the CD34-stained sample and place it on the cytometer.
- Set the fluid control to HI and begin acquiring the sample.
- While viewing the FL1 vs SSC dot plot, increase the FL1 threshold to exclude unwanted debris.

See Figure 4 for examples of plots before and after threshold optimization.

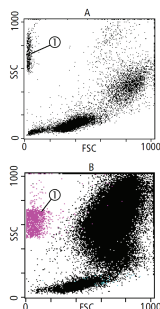
Figure 4 Threshold optimization of leukapheresis sample (1 = debris, 2 = cells, 3 = beads)



- Adjust the FSC gain and SSC gain (if necessary) to ensure all populations are on scale.

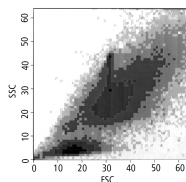
The lymphocyte population should fall between channels 200 and 600 on the x-axis (Figure 5).

Figure 5 FSC vs SSC optimization displays for leukapheresis sample (A) and mobilized peripheral blood (B) (1 = beads)



See Figure 6 for an optimized sample on a non-BD platform. Although FSC is not typically used during analysis, it could provide additional information when gating or identifying stem cells.

Figure 6 Optimized FSC vs SSC plot on a non-BD platform



NOTE Keep the event rate between 100 to 5,000 events per second.

C. Acquisition

- Collect at least 60,000 CD45 events and 1,000 BD Trucount tube beads.

If the sample runs for more than 5 minutes, closely monitor that the tube does not run dry. For more

information, refer to the cytometer user's guide.

NOTE We recommend storing all data in list-mode files.

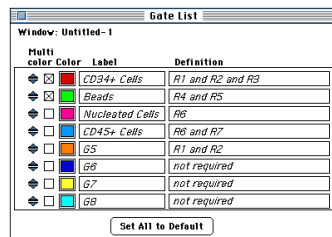
2. When sample acquisition is complete, remove the CD34 tube from the cytometer.
3. Vortex the tube containing the Control-stained sample and place it on the cytometer.
4. Collect at least 60,000 Control events and 1,000 BD Trucount tube beads.
5. Continue acquiring the remaining samples, visually inspecting the threshold for each tube.

Slight adjustments might be necessary, especially for different sample types.

D. Analysis

1. Create the following two-dimensional data displays:
 - nucleic acid dye vs SSC
 - CD45 PerCP vs SSC
 - Two displays of nucleic acid dye vs CD34 PE (the second display will be gated on R1 and R2)
2. Define the following gates as appropriate to your analysis program.

Figure 7 Gate List defining G1 through G5



Gating the CD34 Population

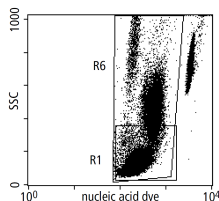
Draw the indicated regions; save all changes to the analysis file after each gate adjustment. Use the same regions to analyze the Control reagent file.

Adjust the regions to reduce nonspecific events as needed. Check that identical regions are used for both CD34 and Control reagent file tags after making adjustments.

3. Start analysis with the CD34 file.

On the nucleic acid dye vs SSC dot plot, draw a generous region (R1) around the lymphocyte population extending into the monocytes but excluding any debris (Figure 8).

Figure 8 Ungated nucleic acid dye vs SSC dot plot (R1 = lymphocytes, R6 = nucleated cells)



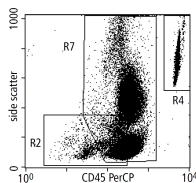
If necessary, extend R1 out to the right. (CD34⁺ stem cells stain brightly with nucleic acid dye.)

NOTE An irregularly shaped region might be required to exclude debris.

4. On the CD45 PerCP vs SSC dot plot, draw a region (R2) around the dim CD45/low SSC population.

Extend the region halfway into the lymphocytes (to include mature stem cells) and into the monocytes (Figure 9).

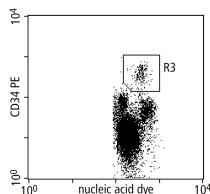
Figure 9 Ungated CD45 PerCP vs SSC dot plot (R2 = dim CD45 cells, R4 = beads, R7 = CD45 cells)



5. Make sure the nucleic acid dye vs CD34 PE dot plot is gated on lymphocytes (R1) and CD45 dim leucocytes (R2) to show only those events that meet the first two criteria for our definition of a stem cell (G5 = R1 and R2).

Draw a region (R3) to include the bright CD34⁺ cells. Avoid extending the region too far down to exclude debris and unwanted cells (Figure 10).

Figure 10 Gated nucleic acid dye vs CD34 PE dot plot; R3 gated off R1 and R2 (R3 = CD34 cells)



Obtaining Bead Counts and Gate Statistics

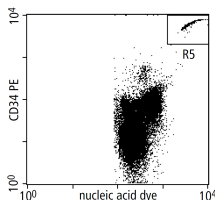
6. On the ungated CD45 PerCP vs SSC dot plot (Figure 9), draw a region (R4) around the bead population.

Keep the left boundary as close to the beads as possible. Be sure to include the last SSC channel.

7. On the ungated nucleic acid dye vs CD34 PE dot plot, draw a region (R5) around the bead population (Figure 11).

This region will exclude any high-SSC granulocytes or eosinophils that might have been included in R4.

Figure 11 Ungated nucleic acid dye vs CD34 PE dot plot (R5 = beads)



Obtaining CD34 as a Percent of Nucleated and CD45 Cell Counts

8. On the nucleic acid dye vs SSC dot plot (Figure 8), draw a region (R6) around the nucleated cell population.

Extend the region to the top of the plot to include the events in the last SSC channel.

9. On the CD45 PerCP vs SSC dot plot (Figure 9), draw a region (R7) around the CD45⁺ population.

Extend the region to the top of the plot to include the events in the last SSC channel.

10. Obtain gate statistics from any of the ungated plots (Figure 12).

Figure 12 Gate statistics

Gate Statistics				
File: algalv15.01				
Sample ID: L.P.M.38				
Patient Name:				
Acquisition Date: 5-Mar-96				
Gated Events: 60000				
X Parameter: FL1-H DNA Dye (Log)				
Log Data Units: Linear Values				
Patient ID: 10				
Case Number:				
Gate: No Gate				
Total Events: 60000				
Y Parameter: FL2-H CD34 PE (Log)				
Gate	Events	% Gated	% Total	
CD34+ cells	1504	2.51	2.51	
Beads	3037	5.06	5.06	
Nucleated Cells	55912	93.19	93.19	
CD45+ Cells	56396	93.99	93.99	
GS	10357	17.26	17.26	

8. RESULTS

Calculation of Absolute Counts

Use the following equation to calculate the absolute CD34 count.

$$(\text{CD34 cells/beads}) \times (\text{BPT/V}) \times \text{DF} = \text{CD34 cells}/\mu\text{L}, \text{ where:}$$

- BPT = beads/tube, which is found on the BD Trucount foil pouch and can vary from lot to lot
- V = test volume

DF = dilution factor
The number of CD34 cells and beads are shown in the Gate Statistics window. In the example shown in Figure 13, the absolute CD34 count is calculated as follows.

$$(1,504/3,037) \times (51,700/50 \mu\text{L}) \times 1 = 512 \text{ CD34 cells}/\mu\text{L}$$

9. LIMITATIONS

- Because of the variability of biological samples, some samples might not be correctly analyzed. An experienced flow cytometrist must critically review all dot plots. An incorrectly high result could lead to an infusate with less than the recommended threshold dose of CD34⁺ cells.
- Laboratories and other users should establish their own normal reference ranges for the BD Procount reagent parameters that could be affected by sex, race, and age of the patient, and preparative techniques. Sex, race, and age of patients should be known when a reference range is determined.

- As for any assay that involves counting statistics, the number of events counted could influence the result.¹⁰ We recommend collecting at least 60,000 events and 1,000 beads for analysis.
- Leukapheresis samples must be stained within 6 hours after collection and run within 24 hours after staining. Peripheral blood and mobilized blood samples must be stained within 24 hours after collection and run within 24 hours after staining. Other samples must be stained within 6 hours after collection and run within 6 hours after staining.
- BD Procount results must be interpreted in conjunction with other information available from clinical evaluation and additional diagnostic procedures.
- Store blood at room temperature (20°C–25°C) before staining.
- Do not use previously fixed and stored patient specimens.
- Performance characteristics have not been established for other sample matrices (cord blood, pediatric samples, bone marrow, and purified product) or on thawed samples.
- BD Procount software might not correctly analyze bone marrow samples.
- The WBC count should not exceed 50.0×10^3 WBC/ μ L and the CD34 cell count should not exceed 2,000 cells/ μ L.

10. EXPECTED RESULTS

Reference Range

Reference ranges for peripheral blood were determined at three geographically distinct sites. Subjects were hematologically normal adults between the ages of 18 and 65 years. No statistically significant differences were found for sites, gender, or age groups, so the data were pooled. See the second Limitation on page 11 for more information about reference ranges. Reference ranges are shown in Table 1.

Table 1 Representative reference ranges in hematologically normal adults

Measures reported	n	Median	SD ^a	95% Reference Range	
				Lower (90% conf. bounds)	Upper (90% conf. bounds)
CD34 cells/ μ L	169	2.2	1.7	0.7 (0.6, 0.8)	6.9 (6.1, 7.9)
%CD34 of nucleated cells	169	0.037	0.025	0.013 (0.011, 0.014)	0.11 (0.095, 0.12)
%CD34 of CD45 cells	169	0.037	0.025	0.013 (0.011, 0.014)	0.11 (0.096, 0.12)

a. standard deviation

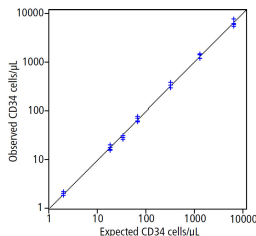
11. PERFORMANCE CHARACTERISTICS

Accuracy

Accuracy was assessed at one site. A leukapheresis sample purified for CD34+ cells by a positive selection technique was analyzed with a hematology counter. The purified sample was spiked into a normal peripheral blood sample at different concentrations. The theoretical count was determined from the dilution factor and the original hematology count, and

compared to results of four replicate BD Procount determinations at each dilution factor. Results are shown in Figure 13.

Figure 13 Regression plot of expected vs observed CD34 cells/ μ L



Within-Specimen Reproducibility

A study was conducted at three sites to assess reproducibility. Ten aliquots (± 1) of four normal peripheral blood samples, two mobilized peripheral blood samples, and five leukapheresis samples were prepared, then acquired. Results are divided into subgroups based on CD34 cells/ μ L. Results are shown in Table 2.

Table 2 Within-specimen reproducibility based on mean CD34 cells/ μ L

Subgroup	n	DF ^a	Mean	SD	CV ^b (%)
CD34 cells/ μ L					
≤ 5 μ L	41	37	3.8	0.5	13
>5 and ≤ 200 μ L	40	36	117	9.9	8
>200 μ L	33	30	332	21.2	6
%CD34 cells of nucleated cells					
≤ 5 μ L	41	37	0.07%	0.002	4
>5 and ≤ 200 μ L	40	36	0.38%	0.031	8
>200 μ L	33	30	1.39%	0.108	8
%CD34 cells of CD45 cells					
≤ 5 μ L	41	37	0.07%	0.010	14
>5 and ≤ 200 μ L	40	36	0.38%	0.028	7
>200 μ L	33	30	1.40%	0.063	5

- a. degrees of freedom
b. coefficient of variation

Stability

A study was conducted to assess specimen stability at one site. Stability was evaluated by comparing baseline samples (stained and acquired within 6 hours) to aged samples (stored for 24 hours unstained, then stored for 24 hours stained for a total of 48 hours) on 10 normal peripheral blood samples, 10 mobilized peripheral blood samples, and 20 leukapheresis samples. Results for each sample type are shown in Table 3.

Table 3 Comparison of baseline and aged samples for normal peripheral blood, mobilized peripheral blood, and leukapheresis samples

Parameter	n	Mean at baseline	Mean change (after 48 hours)
normal peripheral blood			
CD34 cells/ μ L	10	2.16	-0.034
%CD34 of nuc. cells	10	0.046%	0.001%
%CD34 of CD45	10	0.046%	0.001%
mobilized blood			
CD34 cells/ μ L	10	54.3	-1.98
%CD34 of nuc. cells	10	0.288%	-0.002%
%CD34 of CD45	10	0.292%	-0.002%
leukapheresis blood			
CD34 cells/ μ L	20	205	-18.9 ^a
%CD34 of nuc. cells	20	1.06%	-0.079%
%CD34 of CD45	20	1.07%	-0.080%

- a. statistically significant from 0, $p = 0.023$

A follow-up study comparing baseline samples to samples stained and then stored for 24 hours was conducted on 15 leukapheresis samples. Results are shown in Table 4.

Table 4 Comparison of baseline and aged samples (24 hours after staining) for leukapheresis samples

Parameter	n	Mean at baseline	Mean change (after 24 hours)
CD34 cells/ μ L	15	133	6.3
%CD34 of nuc. cells	15	0.656%	0.030%
%CD34 of CD45	15	0.661%	0.031%

Based on these results, we recommend staining peripheral blood samples within 24 hours of collection and acquiring within 24 hours of staining. For optimal results, we recommend staining leukapheresis samples within 6 hours of collection and acquiring within 24 hours of staining. Leukapheresis samples stored unstained for more than 24 hours could show decreased CD34 cells/ μ L.

Comparison of Flow Cytometers

A study was conducted at one site to compare the performance of flow cytometers from two different manufacturers. Normal peripheral blood samples (n = 9), mobilized peripheral

blood samples (n = 9), and leukapheresis samples (n = 12) were run on BD FACSCalibur and Coulter XL flow cytometers. Results are shown in Table 5.

Table 5 Comparison of BD Procount results on BD FACSCalibur instrument (x) vs Coulter XL (y) instrument

Parameter	Slope	Intercept	r
CD34 cells/ μ L	0.92	1.5	0.99
%CD34 of nuc. cells	0.93	-0.0001	0.99
%CD34 of CD45 cells	0.93	-0.0006	0.99

Cross-Reactivity

CD34 has no known cross-reactivity to nonprogenitor cells in blood.

Linearity

The assay is linear from 0 to 2,000 CD34+ cells/ μ L.

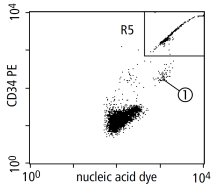
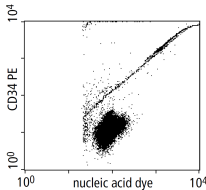
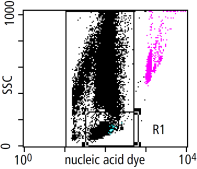
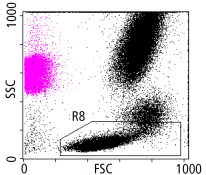
WARRANTY

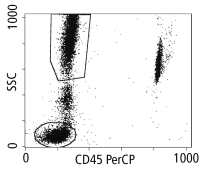
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TROUBLESHOOTING

Problem	Solution
Difficulty determining where to draw the regions	Enlarge the plot for better resolution.

Problem	Solution
A small cluster of events that look like beads appear just below R5 in the nucleic acid dye vs CD34 plot (1). 	High SSC granulocytes/eosinophils can be included in the first bead region (R4). Do not include this population in R5.
Populations appear undercompensated or at a 45° angle.	Set compensation correctly.
Gate statistics don't make sense.	Make sure you are looking at gate stats for the ungated plot, which shows all events.
The FL1 vs FL2 dot plot looks like this before applying the G5 gate: 	1. Create an FSC vs SSC dot plot and place a region around the lymphocytes as shown. 2. Redefine the CD34⁺ cells gate (G1) to include this region (R1 and R2 and R3 and R8). 3. Redefine the G5 gate to include this region (R1 and R2 and R8). NOTE If the FSC vs SSC plot does not look like this example, restrain and run the sample. Optimize FSC vs SSC as described in step 6 on page 8. 
Two clusters of granulocytes can be seen in the FL1-SSC plot. 	The FL1-dim cells are apoptotic or dying granulocytes. They should be included in the nucleated cell count. Set the threshold below these cells and include them in region R6.
Control tube fails QC criteria (see Quality Control on page 6)	1. Make sure the Control-stained sample is the same as the CD34-stained sample. 2. Check your regions and gates. 3. If the Control tube still fails, restrain and rerun the sample.

Problem	Solution
Plot shows poor CD45⁺ resolution. 	Restain sample using fresh reagents or reagents with a different lot number. Contact BD technical support if the problem is not resolved.

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