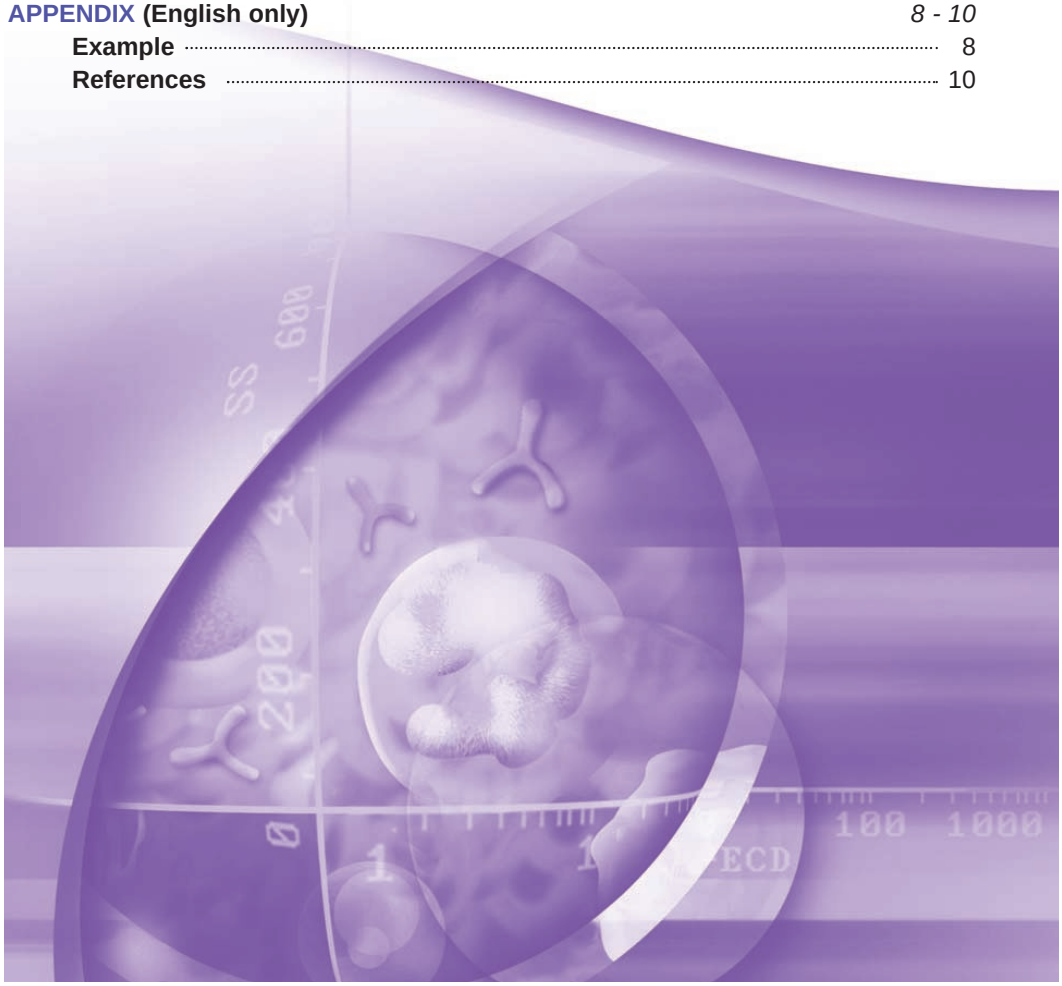


CE IVD REF IM3632

For *in vitro* Diagnostic Use **10 Tests – 20 µL/test – 0.2 mL**

1. INTENDED USE

Stem-Trol™ Control Cells is an assayed quality control product for immunophenotyping analysis using CD45 and / or CD34 monoclonal antibodies reagents and flow cytometry. It provides a positive cell control that is processed in the same manner as a blood specimen when Stem-Trol Control Cells are spiked into a peripheral whole blood sample. This allows verification of reagent performance and the methods used for staining targeted cells (i.e. $CD34^+$ Hematopoietic Progenitor Cells (HPC)), lysing erythrocytes, and analyzing samples with flow cytometry.

Refer to the Stem-Kit™ Reagents (REF IM3630) package insert for complete instructions for use if performing analysis without stemONE™ System Software. Refer to the stemONE System Guide provided with the stemONE System Software (REF 6915452) for complete instructions for automated analysis.

2. SUMMARY AND EXPLANATION

Immunophenotyping analysis using flow cytometry involves the identification and enumeration of targeted cells in biological specimens. The specimens of interest are stained with monoclonal antibodies and erythrocytes are lysed prior to flow cytometric analysis. A positive cell control is required to verify reagent performance, sample preparation methods, and staining procedures (1). A positive cell control should mimic a representative targeted cell in terms of monoclonal antibody performance, erythrocytes lysing, and flow cytometric analysis.

Stem-Trol Control Cells is a liquid preparation of stabilized KG-1a derived cells that have antigen expression and CD45 and CD34 staining properties representative of those found on $CD34^+$ HPC.

3. PRINCIPLE OF TEST

Stem-Trol Control Cells are preserved KG-1a cells that have been modified and stabilized to express the CD34 class III epitopes (2) and the CD45 leukocyte common antigen at densities that approximate normal immature human hematopoietic cells. A mixture of Stem-Trol Control Cells spiked into normal fresh whole blood is first stained with the monoclonal antibody reagents and 7-AAD Viability Dye and then lysed to remove erythrocytes. Stem-Count™ Fluorospheres are added for the direct determination of absolute counts. Flow cytometric analysis of the stained and lysed preparation determines the absolute count of the targeted cells. Expected results are determined on validated and standardized COULTER® EPICS® XL™/ XL-MCL™ flow cytometers using Stem-Kit Reagents (REF IM3630).

4. REAGENT CONTENTS

Stem-Trol Control Cells is suspended in an isotonic solution, containing stabilizers and BSA. The Assayed Cell concentration (cells/µL) is derived from multiple replicate analyses using the reference method. The specific value is located on the vial label

5. STATEMENT OF WARNINGS

1. Specimens, samples, and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
2. Never pipet by mouth and avoid contact of specimens or reagents with skin and mucous membranes.
3. Do not use reagent beyond the expiration date on the vial label.
4. Do not freeze reagents.
5. Do not expose reagent to heat during storage or use.
6. Avoid evaporation and leakage of reagent by capping vials tightly after use, or erroneous results may occur.
7. Stem-Trol Control Cells will settle over extended periods. Ensure that the cells are completely resuspended before use. Avoid excessive mixing to minimize the formation of air bubbles. Do not pipette air bubbles or erroneous results may occur.
8. Use a calibrated positive displacement or repeater pipet to dispense samples and Stem-Trol Control Cells, or erroneous results may occur.

9. Use pipetting techniques recommended by the pipet manufacturer to ensure accurate and precise pipetting of samples and Stem-Trol Control Cells, or erroneous results may occur.
10. Each lot of Stem-Trol Control Cells has a specific concentration of cells. Ensure the correct assayed concentration is used when determining absolute count results.
11. Incubation or mixing times or temperatures other than those specified may give erroneous results.
12. Erroneous results may occur if the flow cytometer is not properly aligned or standardized for fluorescence or if the cell populations are improperly gated.
13. Results determined using flow cytometers, lysing systems, or antibodies that are different from those used to determine the expected results may not fall within the expected range.
14. Use Good Laboratory Practices (GLP) when handling this reagent.

6. STORAGE CONDITIONS AND STABILITY

- Reagent stability: this reagent is stable up to the expiration date stated on the vial label when stored unopened at 2 – 8°C. Do not use after the expiration date. Open vial stability is 30 days. Opened vials must be capped tightly and stored at 2 – 8°C after use. Do not freeze.
- Bring Stem-Trol Control Cells to room temperature (18 – 25°C) prior to use.

IMPORTANT:

Risk of erroneous results if leakage of the Stem-Trol Control Cells occurs. Once opened, Stem-Trol Control Cells must be stored in an upright position to prevent the possibility of leakage. Reagents should not be used if signs of leakage are observed.

6.1 Evidence of Deterioration

Inability to obtain expected results or a shift in light scatter or fluorescence properties may indicate product deterioration. Instrument standardization, sample preparation technique, and antibody performance should also be investigated.

Any change in the physical appearance of Stem-Trol Control Cells (normal appearance is a colorless liquid) or any major variation (>15% change in absolute count) in values obtained from replicated tests by flow cytometry analysis, may indicate deterioration and the reagent should not be used further.

6.2 Reagent Preparation

Stem-Trol Control Cells is a ready-to-use cell suspension. Proper mixing (vortex for 10 – 12 seconds) is required before each first pipetting from the vial. Avoid excessive mixing to minimize formation of air bubbles. Do not pipet air bubbles.

7. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Deionized water.
2. Stem-Kit Reagents (REF IM3630), including 7-AAD viability dye.
3. Ice water bath.
4. Plastic test tubes (12 x 75 mm).
5. Calibrated positive displacement pipet (20 µL, 100 µL) and tips or calibrated repeater pipet (20 µL, 100 µL, 2 mL) and tips.
6. Calibrated standard pipettes (20 µL, 100 µL, 2 mL) and tips.
7. Vortex mixer.
8. Timer.
9. Flow cytometer.
10. stemONE System Software (REF 6915452) ONLY for automated analysis of Stem-Trol Control Cells on COULTER EPICS XL/ XL-MCL flow cytometers equipped with System II™ Software (Version 3.0.)

8. PROCEDURE

For automatic analysis, refer to the stemONE System Guide provided with the stemONE System Software (REF 6915452) and the Stem-Kit Reagents (REF IM3630) package insert for the complete instructions.

When not using the stemONE System Software, refer to the Stem-Kit Reagents (REF IM3630) package insert and the following procedure.

NOTE:

In order to standardize the analysis, when receiving new Stem-Kit Reagents, and at daily intervals afterwards, run a process check by staining Stem-Trol Control Cells with a normal peripheral blood sample from a healthy donor. Furthermore, as Stem-Trol Control Cells are stabilized cells (i.e. nonviable cells), 7-AAD Viability Dye staining may be visually checked on Stem-Trol Control Cells (see the heading: Histogram Creation).

Ensure that the flow cytometer is properly aligned and standardized for fluorescence intensity according to the manufacturer and laboratory guidelines. Ensure that fluorescence compensation settings are properly adjusted according to the manufacturer and laboratory guidelines.
Bring the control reagent and antibodies to room temperature.
For each experiment, label one tube: TROL 45/34/7-AAD.

1. Pipet 20 µL of CD45-FITC / CD34-PE into the tube.
2. Pipet 20 µL of 7-AAD Viability Dye into the tube.

IMPORTANT:
Risk of incomplete lysis if blood specimen remains on the top or side of the test tube. Use care when pipetting to prevent the blood from touching the top or side of the test tube. Clean the tube with a cotton swab, if necessary, to remove all traces of blood specimen from the top or side of the test tube.

3. Into the bottom of the tube, accurately pipet 100 µL of a well-mixed normal whole blood sample using a calibrated positive displacement or repeater pipet.
4. Prepare and add Stem-Trol:
   • Vortex Stem-Trol Control Cells for 5 seconds.
   • Accurately pipet 20 µL of Stem-Trol Control Cells into the tube.
   • Vortex tubes for 5 seconds.
5. Incubate at room temperature (18 – 25°C) for 20 minutes, protected from light.
6. Add 2 mL of prepared 1X NH_4Cl Lysing Solution into tube and vortex immediately for 5 seconds.
   For details on preparing the Lysing Solution, refer to the Stem-Kit Reagents (REF IM3630) package insert.
7. Incubate at room temperature for 10 minutes, protected from light.
8. Store the tubes in a rack placed on ice (2 – 8°C), and protected from light.

IMPORTANT:
Risk of erroneous results if air bubbles are pipetted. Excessive mixing of Stem-Count fluorospheres can create air bubbles. Do not excessively mix the Stem-Count fluorospheres, and do not pipette air bubbles into the sample tubes.

9. Gently mix the Stem-Count Fluorospheres by inverting the vial 3 to 5 times before using. Avoid excessive mixing to minimize air bubble formation.
10. Prior to acquisition, pipette 100 µL of Stem-Count Fluorospheres into the tube.
11. Vortex for 5 seconds immediately after each addition. Store at 2 – 8°C. Repeat vortexing immediately prior to flow cytometric acquisition.

IMPORTANT:
Risk of erroneous results if sample is analyzed more than 1 hour after adding Stem-Count Fluorospheres. Prepared samples must be analyzed within 1 hour of adding Stem-Count Fluorospheres.

8.1 Preparation Summary (Tube Label: TROL45/34/7-AAD)

Reagents & Samples	Volume
CD45-FITC / CD34-PE	20 µL
7-AAD Viability Dye	20 µL
Whole Blood	100 µL
Stem-Trol Control Cells	20 µL
Vortex – Incubate at room temperature for 20 minutes. Protect from light	
1X NH_4Cl Lysing Solution	2 mL
Vortex – Incubate at room temperature for 10 minutes. Protect from light	
Stem-Count Fluorospheres	100 µL

9. MANUAL GATING AND ANALYSIS METHOD

9.1 Protocol Setup

The flow cytometer must be equipped to detect Forward Scatter, Side Scatter and four fluorescence channels. For the FL3 channel (for Stem-Count Fluorospheres monitoring) use a 620 nm band pass filter. For the FL4 channel (for 7-AAD Viability Dye monitoring) use a 675 nm long pass filter.

NOTE:

- **In the following analytical strategy description, instructions for COULTER EPICS XL / XL-MCL brand flow cytometers are specified.**

- **The same gating scheme and series of 8 histograms as stated for specimen analysis, must be followed for Stem-Trol Control Cells. As Stem-Trol Control Cells have size characteristics close to, and express CD45 and CD34 antigens at densities that approximate, normal immature hematopoietic cells, there is no need to modify the region boundaries along the Forward Scatter, Fluorescence 1 and Fluorescence 2 channels. However as Stem-Trol Control Cells Side Scatter characteristics are unique you may adjust the region boundaries along the Side Scatter. Regions A, B, C, and D (see the heading Region Creation for region definition) must be modified to include the Stem-Trol Control Cells characteristic cluster.**

9.2 Histogram Creation

Create histograms as follows:

1. Create Histogram 1 as FL1 CD45-FITC vs Side Scatter.
2. Create Histogram 2 as FL2 CD34-PE vs Side Scatter.
3. Create Histogram 3 as FL1 CD45-FITC vs Side Scatter.
4. Create Histogram 4 as Forward Scatter vs Side Scatter.
5. Create Histogram 5 as FL1 CD45-FITC vs FL2 CD34-PE.
6. Create Histogram 6 as Forward Scatter vs Side Scatter.
7. Create Histogram 7 as Time vs FL3 Stem-Count Fluorospheres.
8. Create Histogram 8 as FL4 7-AAD vs Side Scatter.

Histograms 1 to 4 are intended to characterize $CD34^+$ HPC, a process that may be delayed until the analysis step. These first four histograms are set up according to the ISHAGE Guidelines for $CD34^+$ Cell Determination by Flow Cytometry (2, 3).
Histograms 5 to 7 are intended to monitor parameters that are of importance during the acquisition step. These include the Forward Scatter discriminator, the number of $CD45^+$ events to be collected and the correct fluorosphere singlets accumulation.
Histogram 8 is intended to discriminate and analyze viable events from nonviable events when required.

9.3 Region Creation

Create regions as follows:

1. Histogram 1 – Create a rectilinear Region A to include all $CD45^+$ leukocytes and eliminate platelets, red blood cell debris, and aggregates.
2. Histogram 1 – Create an amorphous Region E on the lymphocytes (bright CD45, low side scatter).
3. Histogram 2 – Create a rectilinear Region B on Histogram 2 to include all $CD34^+$ events with low to intermediate Side Scatter. Set a stop count of 75,000 events ($CD45^+$ events) in Histogram 2.
4. Histogram 3 – Create an amorphous Region C on Histogram 3 to include all clustered $CD45^{dim}$ events.
5. Histogram 4 – Create an amorphous Region D on Histogram 4 to include all clustered events with intermediate side scatter and intermediate to high forward scatter.
6. Histogram 5 – Create a Quadstat Region I on Histogram 5 to verify the lower limit of CD45 expression on $CD34^+$ events.
7. Histogram 5 – Create an amorphous Region H on Histogram 5 to surround all Stem-Count Fluorospheres, including doublets. The region H should be located at the top right corner of the Histogram 5.

NOTE: Be sure that Region H is drawn as an AMORPHOUS region.

8. Histogram 5 – Only on COULTER EPICS XL/XL-MCL flow cytometers equipped with System II Software (version 3.0): Create rectilinear Region K (named " listgate - ") on Histogram 5. Region K boundaries are the first log decade or dual negative region of the two parameter histogram. This Region K allows the elimination of debris during the acquisition in order to analyze 75,000 relevant $CD45^+$ events.
9. Histogram 6 – Copy Region D from Histogram 4 as Region F in Histogram 6.
10. Histogram 7 – Create a rectilinear Region G on Histogram 7 to include the Stem-Count Fluorospheres singlets only. Region G can be labelled as " CAL " to allow automatic calculation of absolute numbers of $CD34^+$ HPC (refer to the instrument manual for further details).
11. Histogram 8 – Create a rectilinear Region J on Histogram 8 to separate viable leukocytes (7- AAD negative events) from non viable events (mainly Stem-Trol Control Cells positive for 7-AAD staining).

9.4 Gate Creation

Create gates as follows:

1. Histogram 1 – Assign"- H" to Histogram 1 to display all events excluding all Stem-Count fluorospheres. Refer to the instrument manual for the creation of " not gates. "
2. Histogram 2 – Assign "A" to Histogram 2 to display all $CD45^+$ events.
3. Histogram 3 – Assign "A" and " B " (AB) to Histogram 3 to display all $CD45^+$ $CD34^+$ events.
4. Histogram 4 – Assign "A" and "B" and "C" (ABC) to Histogram 4 to display all $CD45^+$ $CD34^+$ events clustered events with low to intermediate side scatter and low CD45 staining expression. Events from Region ABCD are real $CD34^+$ HPC.
5. Histogram 5 – Ungated to display all events.
6. Histogram 6 – Assign "E" to display lymphocytes as a visual check on the discriminator.
7. Histogram 7 – Assign "H" to histogram 7 to display all Stem-Count Fluorospheres, including doublets.
8. Histogram 8 – Assign "A" to histogram 8 to display $CD45^+$ events.

9.5 Flow Cytometer Setting

1. Ensure that the flow cytometer is properly aligned and standardized for light scatter and fluorescence intensity according to the manufacturer's and laboratory guidelines. Verify that color compensation is set for standard operation. Refer to the instrument's manual for further instructions.
2. Vortex test tubes for 5 seconds.
3. Perform data acquisition on the flow cytometer. A minimum of 75,000 $CD45^+$ events must be analyzed.
4. Adjust the discriminator and regions by analyzing the TROL 45/34/7-AAD tube.

9.6 Analysis Example

The histograms shown in the APPENDIX (p 66) are displayed in an ascending number order as displayed on the protocol.

9.7 Calculation of $CD34^+$ Stem-Trol Control Cells

Using flow cytometric results automatically adjusted with the Stem-Count Fluorospheres Assayed Concentration obtained using System II Software (Version 3.0) and a COULTER EPICS XL/XL-MCL flow cytometer).

In order to obtain automatically calculated absolute count determinations on COULTER EPICS XL / XL-MCL flow cytometers, the correct Stem-Count Fluorospheres Assayed Concentration must be entered before sample acquisition.

Enter "CAL" as the Name of the Stem-Count Fluorosphere singlets Region G, and enter the value, for example, 1000, into the CAL FACTOR box on the STATISTICS dialog screen of the SET-UP SCREEN PROTOCOL menu.

• Entering the CAL Factor for Stem-Count Fluorospheres:

1. At the Acquisition Run screen, select Setup Screen >> Protocol.
2. Select Statistics >> CAL FACTOR.
3. Enter the CAL factor number (assayed concentration) from the Stem-Count Fluorospheres vial.
4. Press ENTER.
5. Select OKAY.
6. At the prompt, type Y for yes.

When at least 1,000 fluorosphere singlets are acquired, the absolute count for the $CD34^+$ Stem-Trol Control Cells is automatically adjusted and can be taken directly from Region D statistics printout results.

Table (below) :
Example of statistics obtained with tube TROL 45/34/7-AAD on System II Software, using the CAL FACTOR function, where:

- 1024 is the Stem-Count Fluorosphere Assayed Concentration.
- 5248 is the total number of fluorospheres accumulated in the CAL region during the full acquisition time.
  In this example, all counts are adjusted by: 1024/5248.

Stats: Not Normalized Hist		Listgating: Disabled Region ID	Counts Adjusted (Cells/µL)
1	**A**	LEUKS	**9798**
2	**B**	CD34 POS	**279**
3	**C**	CD45 DIM	**264**
4	**D**	Stem-Trol Control Cells	**248**
7	**G**	CAL 1024	**1024**
5	**H**	All Stem-Count Fluorospheres	**1046**

1. Record the adjusted absolute counts (cells/µL) in Regions D from Tubes TROL 45/34/7-AAD.

Example:
Tube TROL 45/34/7-AAD: Stem-Trol Control Cells = 248 cells/µL.

2. Multiply this precise count by the normalization factor (N) = 5.

NOTE:
It is necessary to normalize the absolute count of Stem-Trol Control Cells with regard to the added volumes during the test: Determine N by dividing the volume of the counting reference (Stem-Count Fluorospheres singlets), by the volume of Stem-Trol Control Cells added to the test tube.

Example:
100 µL Stem-Count Fluorospheres are added to 20 µL of Stem-Trol Control Cells:
N = 100 ÷ 20 = 5
Normalized Stem-Trol Control Cells absolute count = 248 x 5 = 1240 cells/µL.

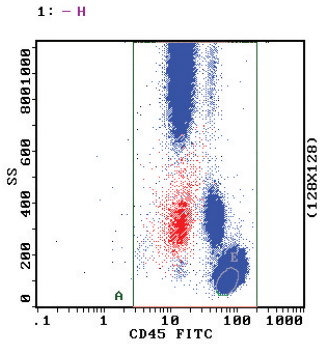
3. Verify the Stem-Trol Control Cells absolute count obtained by comparing it to the Stem-Trol Control Cells.
   Assayed Concentration (cells/µL) stated on the vial label. The Stem-Trol Control Cells absolute count obtained must be within ±15% of the Stem-Trol Control Cells Assayed Concentration. The staining method and lysing process are now validated.

Example:
Stem-Trol Control Cells Assayed Concentration = 1380 cells/µL.
Range of accepted values: 1173 – 1587 (1380 ± 207) cells/µL
The normalized absolute count = 1240 cells/µL is within the range.

IMPORTANT:
If the Stem-Trol Control Cells absolute count falls outside of the range of accepted values, determine whether the pipetting has been correctly performed, especially for the Stem-Count Fluorospheres and Stem-Trol Control Cells. Visually check the flow cytometer reports and the quality of staining and lysing process in your test tubes. Verify the way the region boundaries were set. If necessary, repeat the preparation with new test tubes. Acquire and analyze both the new and the previous series of tubes. Compare the two results. If there is no agreement, call your local Beckman Coulter Representative.

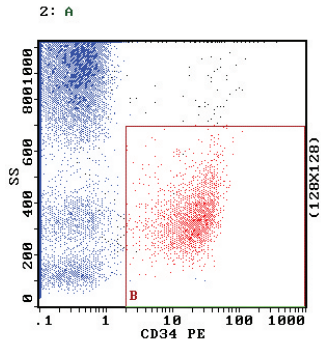
Appendix (English only) p 66 - 68

Example:
The following eight histograms correspond to the analysis of the Stem-Trol Control Cells stained with Stem-Kit Reagents. Below are histograms obtained on a COULTER EPICS XL/XL-MCL flow cytometer equipped with System II Software (Version 3.0).



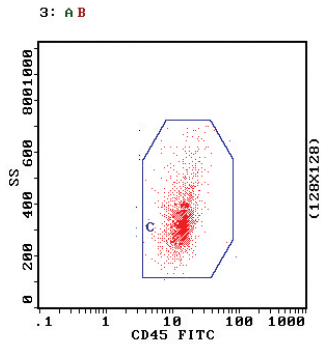
Histogram 1: displays all events minus all Stem-Count Fluorospheres (i.e. "-H" see Histogram 5).

Notes:
• Position Region A to include all $CD45^+$ events (leukocytes) while excluding CD45- debris. Position Region E to include only lymphocytes (bright CD45, low Side Scatter). Region A is intended to serve as an appropriate denominator (total WBC) in the calculation of the percentage of $CD34^+$ HPC.



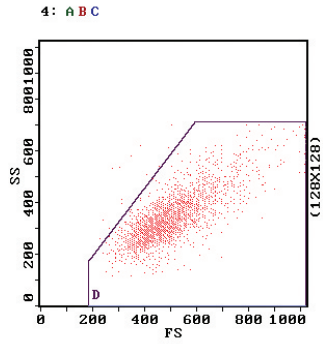
Histogram 2: displays events from region A.

• Adjust Region B to surround $CD34^+$ Stem-Trol Control Cells, including $CD34^{dim}$ events. Upper limit along the y-axis may be set to include $CD34^+$ events with intermediate Side Scatter for $CD34^+$ Stem-Trol Control Cells



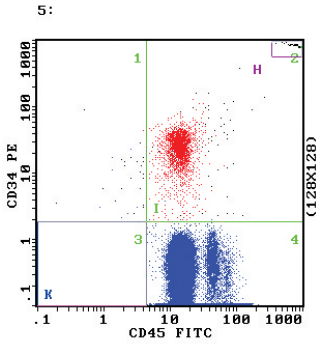
Histogram 3: displays events from A and B.

• Adjust Region C to include cells forming a cluster with characteristic $CD34^+$ Stem-Trol Control Cells (i.e. intermediate Side Scatter and low to intermediate CD45 staining). It is the cluster of events that determines where Region C is centered. Brightly FITC stained events (platelet aggregates and mature monomyeloid cells) must be excluded in the setting of this region.



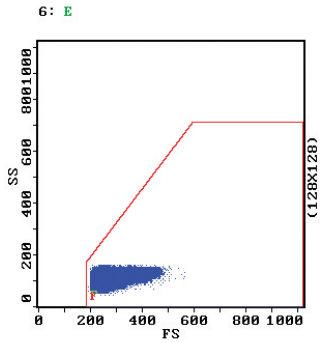
Histogram 4: displays events from regions A and B and C.

• Region D must include events characteristic of Stem-Trol Control Cells. Once the analysis is done, the absolute count of total $CD34^+$ Stem-Trol is given on the statistic printout related to the $CD34^+$ Stem-Trol (Region D).



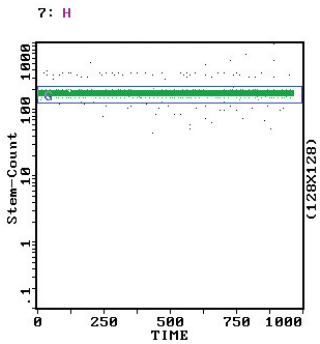
Histogram 5: displays all events.
Important: This histogram is useful to visualize the lower limit of CD45 expression within the $CD34^+$ events.

- Set Quadstat Region I2 to enclose all $CD45^+$ $CD34^+$ events. Set the left boundary of Quadstat Region I2 to include all $CD34^+CD45^{dim}$ events. Verify on Histogram 1 that the lower limit of Region A includes all $CD34^+$ events (use Histogram 5 as a guide).
- Amorphous Region H is drawn to include all Stem-Count Fluorospheres. Region H should be located at the top right corner of the Histogram including the last channel of the FL1 Log (on the right) and FL2 Log scales (on the top).
- Set Region K (named " listgate - ") to exclude most of the double negative events from the acquisition.



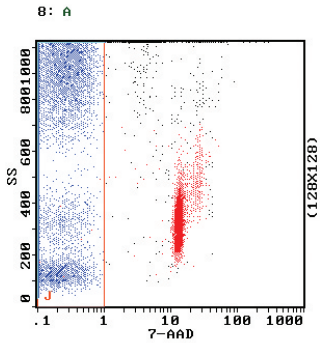
Histogram 6: displays events from region E.

- Verify whether the Forward and Side Scatter gain parameters of the flow cytometer are optimally set for the processed sample. If necessary, adjust the Forward Scatter voltage/gain so that the smallest lymphocytes scatter in the middle-left part of the histogram. Adjust the Forward Scatter to ensure that even the smallest lymphocytes scatter above the discriminator.



Histogram 7: displays events from region H.

- Region G encloses the fluorosphere singlet population only. Check that the fluorosphere singlets accumulate homogeneously and constantly over time.
- Label Region G as "CAL" to allow automatic calculation of absolute numbers of $CD34^+$ Stem-Trol Control Cells. Type the correct assayed concentration of the current batch of Stem-Count Fluorospheres (refer to section 9.7 of this document or to the instrument manual for further details).



Histogram 8: displays events from region A.

- For Stem-Trol Control Cells analysis in the presence of 7-AAD Viability Dye, Histogram 8 allows you to visually check the 7-AAD Viability Dye positive staining. Region J is not used for Histograms 1, 2, 3, and 4.

References

1. National Committee for Clinical Laboratory Standards. Clinical Applications of Flow Cytometry: Quality Assurance and Immunophenotyping of Lymphocytes; Approved Guideline, NCCLS Document H42-A, [ISBN 1-56238-364-7]. Vol. 18, N° 21. January 1999 Edition.

2. Roth, P., Maples, J., Hall, J., Dailey, T.,"Use of control cells to standardize enumeration of CD34+ stem cells", 1996, Ann. NY Acad. Sci., 770, 370-372.

3. Gratama, J.W., Keeney, M., and Sutherland, D.R., «Enumeration of Hematopoietic Stem and Progenitor Cells», Current Protocols in Cytometry, 1999, 6.4.1-6.4.22.

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