APPLICATION NOTE

FLOW CYTOMETRY

CyAn™ ADP | CD34 Enumeration

Single-platform flow cytometry absolute cell counting protocols provide increased robustness for CD34+ cell enumeration by limiting potential sources of imprecision. However, samples with any cellular fragmentation or debris provide challenges for these rare cell event assays. We have developed a single platform CD34+ cell counting kit that utilizes the ISHAGE gating protocol and 7-AAD to eliminate dead cells. We demonstrate the use of the kit on a CyAn ADP LX 9 Color flow cytometer.

Materials and Methods

Samples should be stained in duplicate to confirm the results. A leukapheresis sample from a growth factor stimulated patient was used in this procedure for demonstration purposes. Into each of two tubes, 100 µL of sample was added by wet tip reverse pipetting, gently wiping the tip to remove excess fluid. Add 10 µL of the dual color CD45/CD34 RPE reagent. After reagent addition, the tubes were gently vortexed and incubated for 15–30 minutes at room temperature. After incubation, 2 mL of diluted EasyLyse™ was added to each tube. The tubes were gently vortexed and incubated at room temperature in the dark for 10 minutes. If viability staining is required, add 10 µL of 7-AAD and incubate for 5 minutes. After incubation, 100 µL of CytoCount™ bead solution was added to each tube. It is important that the beads are very carefully mixed before pipetting a sample (do not vortex since this introduces air bubbles). Pipette with wet tip, reverse pipetting gently wiping the tip before ejecting the bead solution. Gently vortex the tubes. The samples should be run within 45 minutes after adding the EasyLyse.

Results

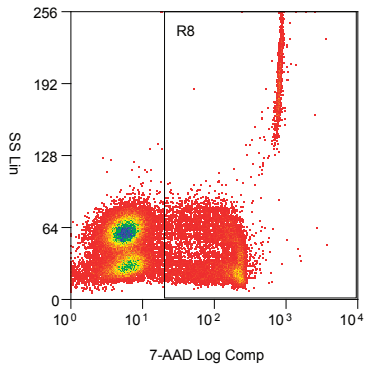
When running these samples on the CyAn ADP LX 9 Color, a CD45 FITC trigger was used, and the threshold was adjusted to eliminate CD45- events. The full gating protocol employed is described in more detail in the CD34 Count Kit product insert. The gating strategy discussed below is critical for calculating the viable CD34+ cell count/µL. This gating strategy employs 7-AAD to eliminate all non-viable cells. The absolute number of CD34 cells (per µL) can be calculated using the following equation:

$$\frac{\left(\text{Number of events in Figure 5B region R4}\right) \times \left(\text{CytoCount Bead concentration/µL}\right)}{\text{Number of beads in Figure 7 region R}}$$

Discussion

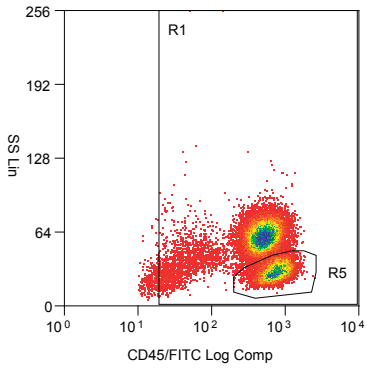
As shown below, this cell preparation and analysis is a simple and quick way to get an accurate viable absolute CD34 count. The critical step in the procedure is the precise pipetting of the sample and the CytoCount beads. A lyse no wash procedure must also be used to minimize cell loss.

Figure 1



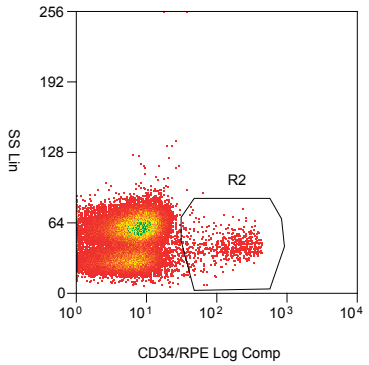
7-AAD vs SSC and R8 defines all non-viable cells. The gate "not R8" is applied to the following histograms.

Figure 2



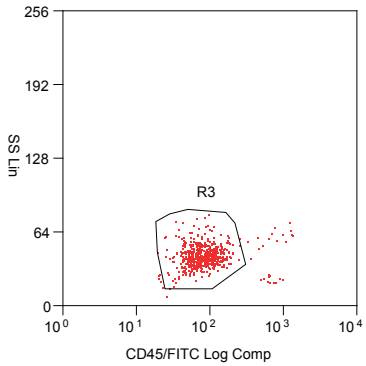
Region R1 is used to define the CD45+ population and region R5 the lymphocyte population.

Figure 3



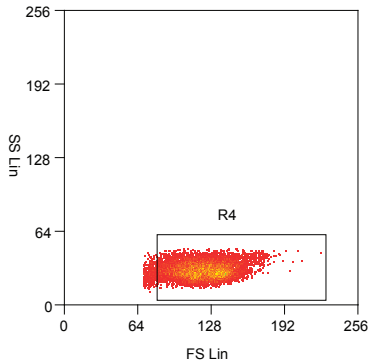
CD34 RPE vs SSC, region R2 is used to define the CD34+ cells.

Figure 4



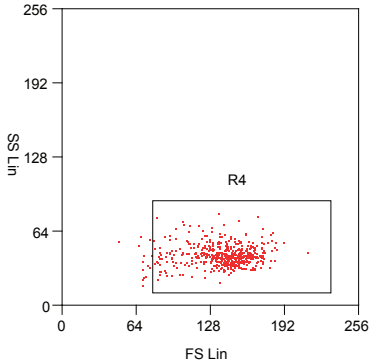
CD45 FITC vs SSC gated through the gate R1 and R2, Region R3 is created to define the CD34 events that are CD45^{dim}.

Figure 5a



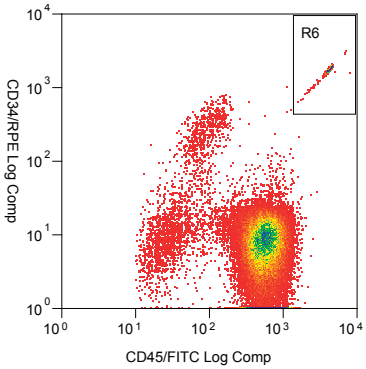
FSC vs SSC gated from the lymphocyte gate (R5). A region R4 is created and adjusted so that the lowest FSC position is equal to the smallest lymphocyte.

Figure 5b



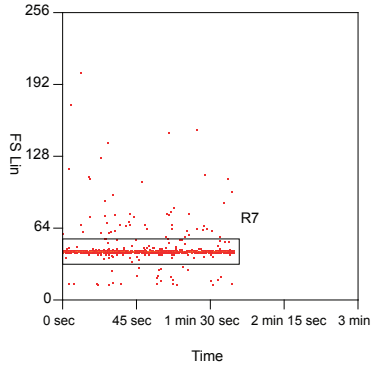
FSC vs SSC gated from the gate R1 and R2 and R3. The events shown in the region R4 are the number of viable CD34 events.

Figure 6



CD45 FITC vs CD34 RPE ungated. A region R6 is created to define the CytoCount beads.

Figure 7



Time vs FSC gated through R6. The region R7 is created to define the CytoCount singlet bead population.

TECHNICAL TIPS

- **Kit Storage.** It is important to make sure that the CytoCount vial is stored in an upright position to prevent fluid leakage
- **During Preparation.** Re-suspend the CytoCount beads by gently rotating the vial (*e.g.,* a blood turner). Harsh vortexing could introduce air bubbles into the fluid.

  Use the same calibrated pipette for the sample and the CytoCount beads to ensure an accurate count.
- **Before Running Samples.** Verify that the instrument has the appropriate warm-up time and is aligned using a standard bead particle.
- **During Acquisition.** In order to have a statistically significant count of the CD34+ cells a minimum of 100 CD34 events and at least 1000 CytoCount beads should be counted.

References

1. Brando B, Barnett D, et al. 2000. Cytofluorometric methods for assessing absolute numbers of cell subsets in blood. *Communications in Clinical Cytometry 42:327-46.*

PRODUCT	CODE
CyAn ADP LX 9 Color	CY201
CD34 Count Kit	K2370

For research use only – not to be used in diagnostic procedures.

The protocols in this application note might deviate from the normal recommended protocol/specification guidelines which are included with the Dako product or any other non-Dako product.

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