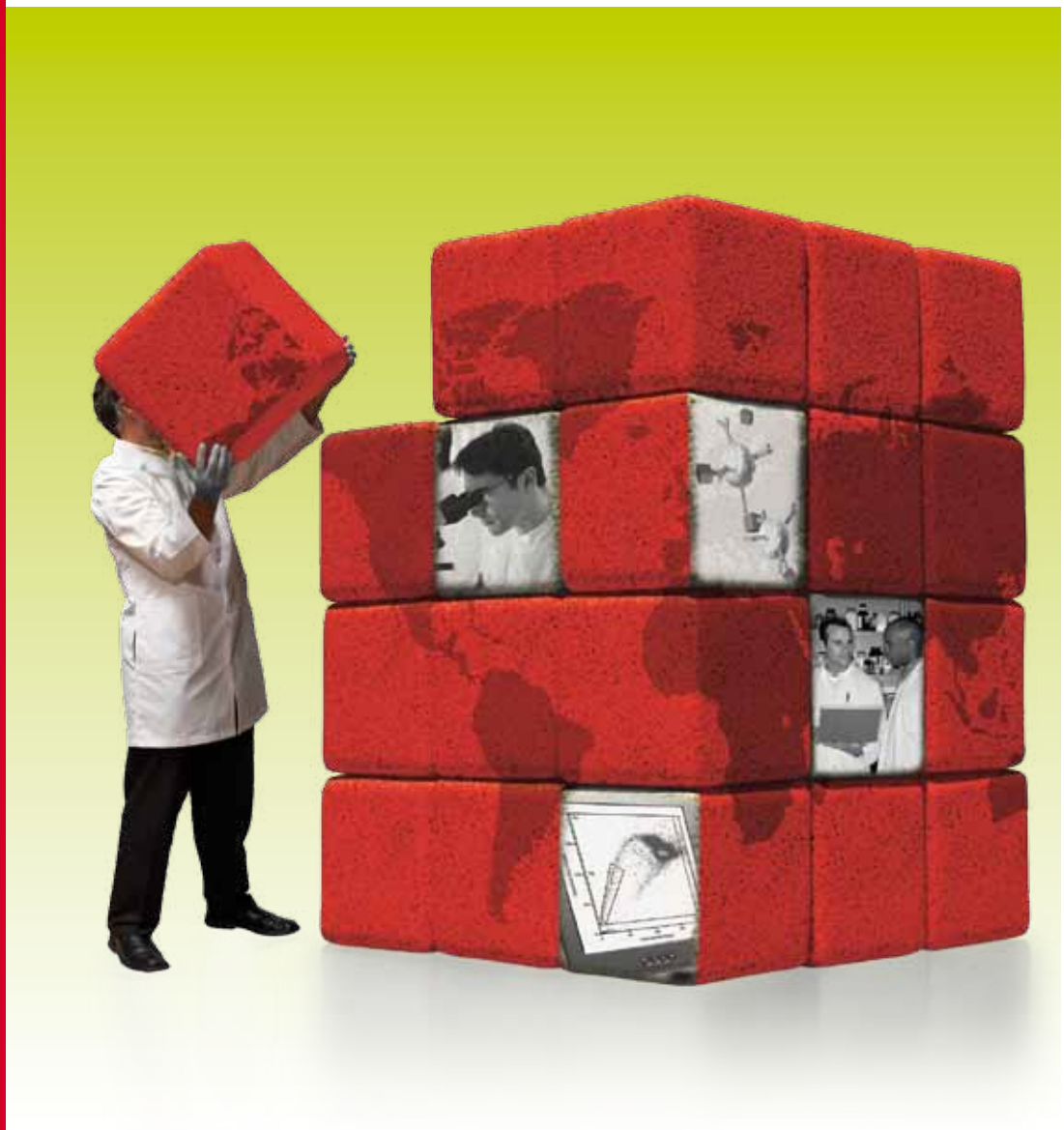
Dako

THE SHAPE OF EXCELLENT
LABORATORY PERFORMANCE

CD34 | Count Kit



A Reliable Kit for Absolute Count of Viable CD34+ Cells

- Compatible with the ISHAGE protocol
- Single platform method
- High accuracy
- Reduced sample handling
- For *in vitro* diagnostic use in flow cytometry

Not for sale in the US

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To maximize quality

Background

Hematopoietic cell transplantations are used in the treatment of blood disorders, malignancies and genetic abnormalities.

Bone marrow was initially the source of choice for transplantation, however, the use of peripheral blood stem cells (PBSC) to reconstitute hematopoiesis has increased tremendously over the last decade.

The success of the transplant is crucial depended on the total number of CD34 cells in the donor material. Conventionally, the CD34 cell count is approximated by a two platform method, using a hemacytometer to determine the total white blood cell count, and a flow analysis where the CD34 cell count is determined relative to the white cell count (Described in more detail in the package insert, and references within).

The CD34Count kit offers a method to obtain the total number of CD34 positive cells in human mobilized peripheral blood samples and leukapheresis samples, applying only a single flow cytometry test.

Enumeration of CD34+ Cells

As flow cytometry provides a rapid and simultaneous analysis of both quantitative and qualitative properties of CD34+ cells, this method has become an important tool for the monitoring of “on-line” yield of CD34+ cells and for the determination of the optimal time for harvesting.

However, the variation in the enumeration of CD34+ cells by flow cytometry is significant due to different gating strategies and the use of different monoclonal antibodies, fluorochromes and lysing solutions. Thus, standardized guidelines are important for obtaining reliable and comparable results.

Over the years, several guidelines such as Milan, Nordic and ISHAGE have been created in the endeavor to standardize the enumeration of CD34+ cells. Today, the majority of users recognizes the ISHAGE single platform protocol as the “golden standard”.

A Reliable Kit for Absolute Count of Viable CD34+ Cells

In order to obtain accurate, reliable and reproducible CD34+ counts, Dako has developed the CD34Count Kit which complies with the ISHAGE single platform protocol.

The kit applies the four-parameter strategy of the above protocol and includes two antibodies, anti-human CD34 and anti-human CD45. Furthermore, it contains CytoCount™, a suspension of fluorescent microspheres, which is used as a reference population for the calculation of the absolute CD34+ value.

In addition, the kit includes EasyLyse™, a no-wash, fixative-free, ammonium chloride red-blood-cell lysing reagent, and the viability dye, 7-aminoactinomycin D (7-AAD), to exclude dead cells from the analysis.

High Accuracy

To minimize potential sources of inaccuracy, all the results used in the CD34+ cell calculation derive from a single platform, the flow cytometer.

To increase the accuracy of the enumerated viable CD34+ cells in the sample even more, the CD34Count Kit employs the practice of:

- Exclusion of washing and fixing procedures of the sample to avoid cell loss
- Identification and quantitation of viable CD34+ cells by use of the viability dye, 7-aminoactinomycin D

Optimized Analysis

As the CD34Count Kit utilizes the ISHAGE single platform protocol, the absolute number of CD34+ cells is directly derived from a single flow cytometric measurement. This optimizes the analysis by:

- Reducing the sample handling
- Minimizing the workload
- Reducing the cost of the test compared to conventional platform counting

User-Friendly

The CD34Count Kit is simple to use and is provided with detailed instructions. Vials are numbered according to the order of use.

The kit has been designed and optimized for the ISHAGE guidelines for single platform.

Main steps in procedure:

1. Pipette 100 µL of the sample into a test tube using reverse pipetting.
2. Add 10 µL antibody mixture (anti-CD45/FITC + anti-CD34/RPE) and incubate for 15 min.
3. Add 2 mL EasyLyse™ (the lysing solution) and incubate for 10 min.
4. Add 10 µL 7-AAD and incubate for 5 min.
5. Add 100 µL CytoCount™ (the reference beads) using reverse pipetting, applying the same pipet as used to pipette the sample.
6. Analyze on the flow cytometer and calculate the absolute viable CD34+ cells using the single platform ISHAGE protocol.

For details please see instructions for use for K2370.

Enumeration of CD34+ Cells (example)

CD34Count Kit is applied on a leukapheresis sample, and the ISHAGE gating protocol is used for determination of the absolute number of viable CD34+ cells.

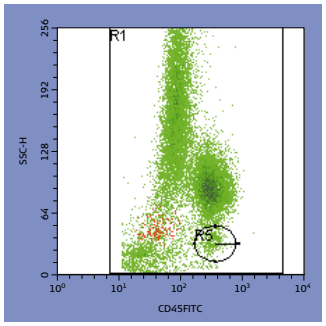


Figure 1. *CD45/FITC vs. SSC showing all events excluding dead cells and beads. R1 is set around the CD45-positive events. R5 is set around the lymphocytes.*

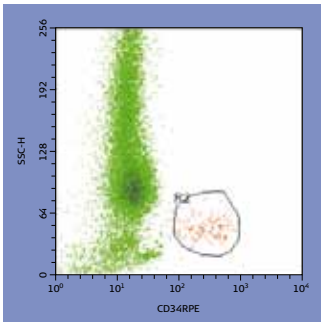


Figure 2. *CD34/RPE vs. SSC. The plot is gated on a logical gate including R1 and not R8 (the dead cells and reference beads). R2 is set around CD34+ cells.*

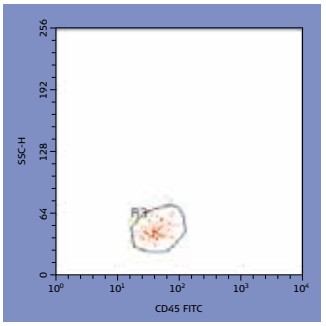
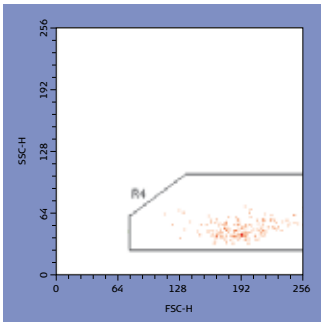


Figure 3. *CD45/FITC vs. SSC gated on a logical gate including R1, R2 and not R8. True CD34 events form a discrete cluster, and a region is set around this population (R3).*



Region	Count	% ...	% All
Total	216	100.00	0.37
R4	216	100.00	0.37

Figure 4. *FSC vs. SSC gated on a logical gate including R1, R2, R3 and not R8. R4 illustrates the CD34+ progenitor cells. The counted number from R4 is used to calculate the absolute CD34+ cell number.*

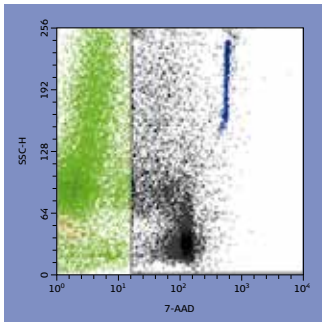


Figure 5. *7-AAD vs. SSC. R8 is set around the 7-AAD-positive cell events.*

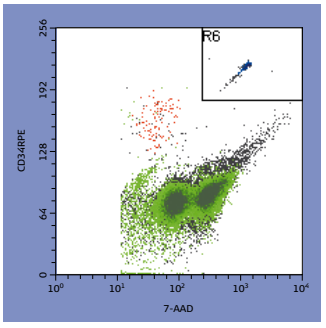
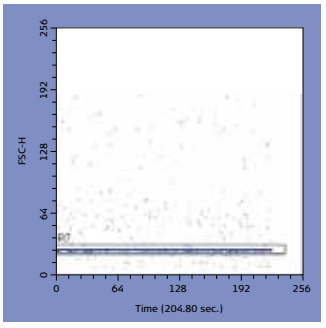


Figure 6. *CD45/FITC vs. CD34/RPE. R6 is set around the CytoCount™ beads.*



Region	Count	% Hist	% All
Total	3498	100.00	5.98
R7	3276	93.65	5.60

Figure 7. *Time vs. FS gated on the R6 events, the CytoCount™ beads (see also Figure 6). The counts from R7 are used for calculating the absolute CD34+ number.*

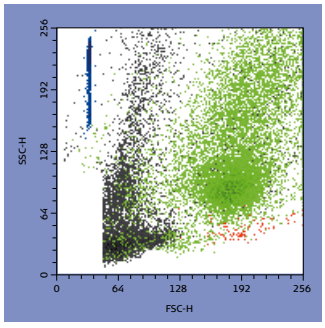


Figure 8. *FSC vs. SSC ungated.*

Calculation

Absolute concentration of CD34+ cells:

$$\frac{\text{Counted number of CD34+ cells x CytoCount™ concentration x Dilution factor}}{\text{Counted number of CytoCount™ beads}}$$

CD34+ concentration:

$$\frac{216 \times 1006 \times 1}{3276} = 66 \text{ CD34+ cells/µL}$$

Kit Contents

Vial 1	Monoclonal Mouse Anti-Human CD45/FITC, Clone T29/33, and Monoclonal Mouse Anti-Human CD34/RPE, Clone BIRMA-K3 1 mL, ready-to-use
Vial 2	EasyLyse™ Erythrocyte-lysing reagent, ammonium-chloride based 2 x 5 mL, 20 x concentrated
Vial 3	7-Aminoactinomycin D (7-AAD) For viability staining 1 mL (0.01% w/v), ready-to-use
Vial 4	CytoCount™ Count control beads 17 mL, ready-to-use after resuspension

Product	Package Size	Code	Regulatory status EU
CD34Count Kit	50 duplicate tests	K2370	CE – IVD*

* Complies with Directive 98/79/EC of the European Parliament and of the Council on *in vitro* diagnostic medical devices.

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