CD34-positive stem cell counts are important in monitoring stem cell transplantation. The Dako CD34Count Kit is an ISHAGE-compatible protocol that provides highly accurate results and reduced sample handling.
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 crucially dependent 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 cytometer to determine the total white blood cell count, and a flow analysis where the CD34 cell count is determined relative to the white blood 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, using 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 a preferred method.

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.

Main steps in the procedure

1. Pipette 100 μL of the sample into a test tube using reverse pipetting.
2. Add 10 μL antibody mixture (anti-CD45/RTC + 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 pipette 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.
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.

**Calculation**

**Absolute concentration of CD34+ cells:**

\[
\text{Counted number of CD34+ cells} \times \frac{\text{CytoCount™ concentration}}{\text{Dilution factor}} \times \frac{\text{Counted number of CytoCount™ beads}}{1}
\]

**CD34+ concentration:**

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

* Concentration stated on label of CytoCount™
**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

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**ORDERING INFORMATION**

<table>
<thead>
<tr>
<th>Product</th>
<th>Size</th>
<th>Code</th>
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<tbody>
<tr>
<td>CD34Count Kit</td>
<td>50 duplicate tests</td>
<td>K2370</td>
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The CD34Count kit offers a method to obtain the total number of CD34-positive cells in human mobilized peripheral blood samples and leukapheresis samples, using only a single flow cytometry test.

Dako has a comprehensive portfolio of more than 220 reagents for flow cytometry, including CD1a, CD90, CD117 and TdT, commonly used for stem cell analysis.