## Counting Nucleated Cells from Whole Blood

Nucleated blood cell concentration is a critical parameter in immunology research. The NucleoCounter® instruments are a rapid, cost-effective alternative to conventional hematology analyzers, using red blood cells lysis and dual-fluorescence nuclear staining to measure the nucleated cell count and viability in whole blood. The analysis itself only takes 50 seconds, and doesn't require instrument warm-up or calibration. Thus the NucleoCounter® is the ideal instrument for research and bioprocessing laboratories with moderate sample processing needs. In contrast to hematology analyzers, the NucleoCounter® can be used more broadly for counting cultured cells, with unparalleled precision.

## Fast and Cconvenient Determination of Cell Count and Viability

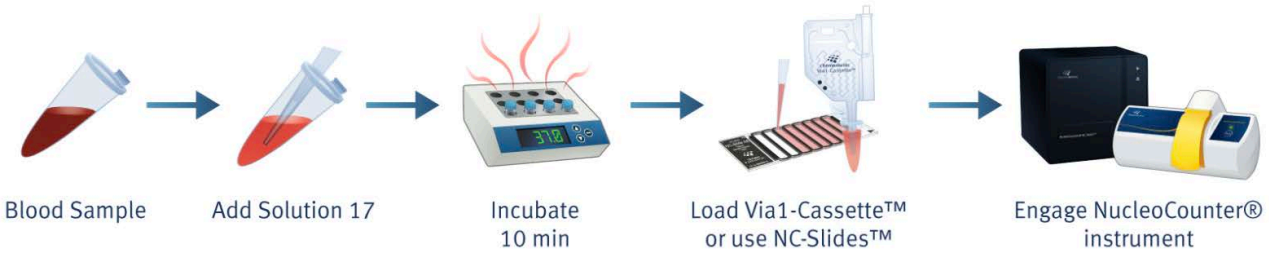


**Figure 1: Fast Determination of Cell Count and Viability of Nucleated Cells from Whole Blood.** Quickly incubate the blood sample with Solution 17 and directly load the Via1-Cassette™ or NC-Slide™ for analysis using the NucleoCounter® instruments.

Hematology analyzers are often used to count and characterize blood cells; however they can be complex and expensive to operate. The NucleoCounter® instruments offer an easy and fast protocol to precisely determine viability and cell count directly from whole blood. Adding Solution 17 to a blood sample followed by a short incubation time lyses the erythrocytes allowing the diluted blood sample to be analyzed by the NucleoCounter® instruments using the Via1-Cassette™ or the NC-Slides™ (Figure 1). NucleoCounting is fast and inexpensive, and can be used for a broad range of counting applications, e.g. in adoptive immunotherapies and for counting bone marrow-derived mesenchymal stem cells.

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## Counting Nucleated Cells in Blood

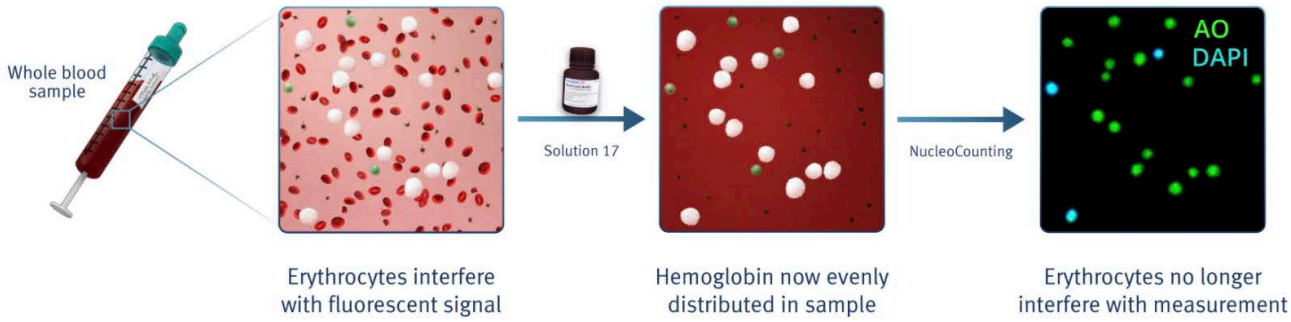


**Figure 2: Precise Counting of Nucleated Cells Directly from Whole Blood Samples.** By adding Solution 17 -an ammonium chloride containing buffer – to the blood sample and allowing for a short incubation, erythrocytes will be lyzed, leaving only the nucleated cells to be stained with Acridine Orange (all cells) and DAPI (dead cells). Afterwards, the nucleated cells can be reliably detected using the NucleoCounter® instruments.

The whole blood sample is mixed with Solution 17, a blood lysis buffer. Solution 17 contains ammonium chloride, leading to swelling and osmotic rupture of erythrocytes [3], and some extra dye of Acridine Orange to compensate the quenching effect of the hemoglobin. After a short incubation time, the sample is stained with the two fluorescent dyes Acridine Orange and DAPI for analysis, e.g. using the pre-loaded Via1-Cassette™ and the NucleoCounter® NC-200™ (Figure 2). Acridine Orange will stain all cells green and DAPI will only stain the dead cells blue. For determination of viability and cell count, the NucleoCounter® instruments will automatically perform fluorescent image acquisition and calculate the results. The NucleoView™ software allows for easy coupling between the obtained image and scatter plots showing quantitative fluorescence for precise control of the analysis.

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## Importance of Nucleated Cell Count in Blood

Blood samples are often used as starting material in life science and contain a mixture of different cells, e.g. lymphocytes, monocytes, granulocytes, hematopoietic stem cells as well as erythrocytes and platelets. The nucleated cell fraction is very interesting for scientists working in vaccine and assay development, transplant immunology and cancer research[1,2]. As the isolation procedure of different subpopulations from blood is usually time-consuming and expensive, the NucleoCounter® instruments can determine the number of nucleated cells prior to and throughout the isolation process for routine monitoring. The quality of blood samples after shipping or storage can be evaluated and it is possible to use the NucleoCounter® instruments for assay readout in pre-clinical animal models and for determining cell count from bone marrow aspirates.


**Application Note (PDF): Viability and Cell Count Whole Blood Assay**

1. Zhang, M. and B. Huang, The multi-differentiation potential of peripheral blood mononuclear cells. Stem Cell Research & Therapy, 2012. 3(6): p. 48-48.

2. Park, B., K.H. Yoo, and C. Kim, Hematopoietic stem cell expansion and generation: the ways to make a breakthrough. Blood research, 2015. 50(4): p. 194-203.

3. Crippen TL, et al., Analysis of salmonid leukocytes purified by hypotonic lysis of erythrocytes. Journal of Aquatic Animal Health, 2001. 13: p. 234-245.

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