Application note No. 0261. Rev. 1.1

NucleoCounter® NC-250™

Whole Blood - Viability and Cell Count using NC-Slides

Product description

The NucleoCounter® NC-250™ system enables the user to perform automated cell counting and viability analyses of mammalian leukocytes in blood samples containing erythrocytes.

Application

Solution 17 used together with the NucleoCounter® NC-250™ facilitates determination of viability and concentration of leukocytes in samples containing red erythrocytes by measuring cell counts (total and non-viable) per volume. The NC-Slide A2™ enables measurements of 2 blood samples at the same time with a high precision, whereas the NC-Slide A8™ enables measurements of up to 8 blood samples at the same time with a moderate precision. The NC-Slide is for one-time-use only, and we strongly recommend discarding the slide after use even in cases where not all chambers have been used. Incubation of the sample at 37°C for 10 minutes in Solution 17 lyses the erythrocytes and dilutes the hemoglobin present in blood sample that quench the fluorescence light. The assay has been optimized to analyze leukocytes from ordinary blood samples.

Introduction

In order to determine viability and count of leukocytes, a sample is diluted with Solution 17 and incubated at 37°C for 10 minutes. Solution 17 contains two different dyes, staining the entire population of leukocytes and the non-viable leukocytes, respectively. After loading the NC-Slide it is placed in the NucleoCounter® NC-250™ where cell concentration and viability are determined.

The nominal depth of the chambers in the NC-Slide is 100 µm, with 90 % of all chambers being in the range from 90-110 µm.

Procedures

Materials needed

- Blood sample to be counted
- NC-Slide A2™ or NC-Slide A8™
- Heating block
- Solution 17

1. The blood sample is mixed to obtain a homogenous suspension. Pipette a representative sample from the blood sample into a microcentrifuge tube.
2. Add 9 volumes of the Solution 17 to the microcentrifuge tube with the blood sample. E.g., if the volume of the blood sample is 20 µl then add 180 µl of Solution 17. Mix by pipetting.
3. Incubate at 37°C on a heating block for 10 minutes. Optionally, the incubation time can be reduced 3 minutes. However, this will decrease the robustness of the assay. Do not exceed 30 minutes as this will affect the viability.
4. Load ~30 µl or ~10 µl of each sample into the chambers of the NC-Slide A2™ or NC-Slide A8™, respectively. Place the loaded slide on the tray of the NucleoCounter® NC-250™ and select "**Viability and Cell Count – Blood Assay**" and sample unit **NC-Slide A2™** or **NC-Slide A8™** and press RUN.

After analysis the viability (in percent) and the concentrations (cells/ml) of all and non-viable leukocytes are displayed. The cell concentrations have been compensated for the dilution caused by the addition of Solution 17. If the cell sample has been further diluted or concentrated and the user has entered the volumes into the user interface the dilution factor has also been taken into account and the returned cell concentration is for the original cell concentration.

Gydevang 43 • DK-3450 Allerod-Denmark • Phone: (+45) 48 13 10 20 • Fax: (+45) 48 13 10 21 • Support: contact@chemometec.com • Web: www.chemometec.com

Notes

To assure reliable results, it is recommended that the cell concentration of the counted leukocytes should be in the range of $5 \cdot 10^4$ cells/ml to $5 \cdot 10^6$ cells/ml. If the cell concentration is above $5 \cdot 10^6$ cells/ml, the cell suspension can be diluted with PBS to achieve the desired concentration. The diluted cell sample is then treated as described above. By inserting the value for the dilution volume in the dilution field on the user interface the returned cell concentration is for the original cell sample.
If the concentration of cells is below $5 \cdot 10^4$ cells/ml then the cell concentration may be increased by centrifugation followed by resuspension of the pellet using PBS. The resuspended cell sample is then treated as described in the procedure. By inserting a negative value representing the volume removed from the sample in the dilution field on the user interface the returned cell concentration is for the original cell sample.

Viability

The viability is calculated as follows:

$$\% \text{ viability} = \frac{Ct - Cnv}{Ct} \cdot 100\%$$

% viability	The percentage of viable cells in the sample
C_t	The total concentration of cells
C_{nv}	The concentration of non-viable cells

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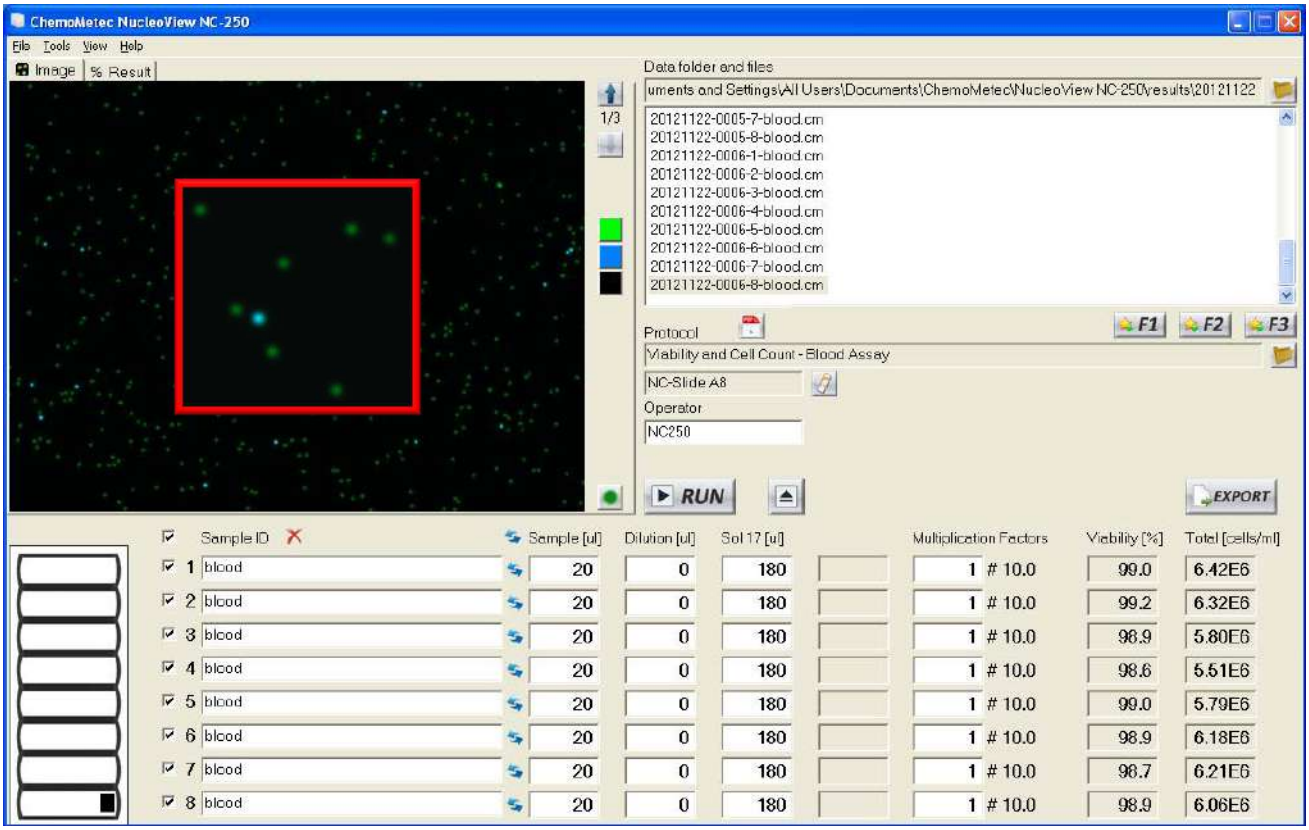

Determination of count and viability of leukocytes in whole blood samples. The blood sample was diluted 10 times with **Solution 17** and incubated at 37°C on a heating block for 10 minutes. All leukocytes are stained with acridine orange and appear green while non-viable leukocytes are stained with DAPI and appear blue. An insert shows a close up of a part of the image. The results are presented at the bottom right and extended results are presented in the result tab page.

Troubleshooting

Inaccurate and imprecise counting:

When setting up a new cell line it is important to inspect that the cell line is counted correctly. The cells included in the total count can be marked by clicking on the overlay button in the bottom right corner of the image. Visual inspect the image to evaluate in the vast majority of the cells has been counted correctly. If this is not the case right click on the image file in question and choose “Show Data in Plot Manager”. Inspect the gates displayed in the Plot Manager. If the gating is inappropriate right click on the image file in question again and choose “Start Protocol Adaptation Wizard”. Adapt the gate(s) to cover the cell population (do not include debris and very large objects) and save the changes to a new protocol. Note that the user is responsible for defining appropriate gating of the particular cell line.

Inappropriate loading of the NC-Slides:

Due to variations in chamber volumes the exact amount needed to fill the chamber may vary. Make sure that the chamber is completely filled and that no excess liquid spreads into other chambers or onto the top of the coverslip. Furthermore, avoid introduction of air bubbles into the chambers. Insufficient filling and air bubbles may cause cell movement compromising the quality of the image analysis.

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Handling and storage

For handling and storage of ChemoMetec instruments and reagents, cassettes and NC-Slides refer to the corresponding product documentation. For other reagents refer to the material data sheet from the manufacturer of the reagents and chemicals.

Warnings and precautions

For safe handling and disposal of the ChemoMetec reagents, cassettes and NC-slides refer to the corresponding product documentation and the NucleoCounter® NC-250™ user's guide. For other reagents refer to the safety data sheet from the manufacturer of the reagents and chemicals required for this protocol. Wear suitable eye protection and protective clothes and gloves when handling biologically active materials.

Limitations

The NucleoCounter® NC-250™ system is FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE. The results presented by the NucleoCounter® NC-250™ system depend on correct use of the reagents, NC-slide and the NucleoCounter® NC-250™ instrument and might depend on the type of cells being analyzed. Refer to the NucleoCounter® NC-250™ user's guide for instructions and limitations.

Liability disclaimer

This application note is for RESEARCH PURPOSES ONLY. It is not intended for food, drug, household, or cosmetic use. Its use must be supervised by a technically qualified individual experienced in handling potentially hazardous chemicals. The above information is correct to the best of our knowledge. Users should make independent decisions regarding completeness of the information based on all sources available. ChemoMetec A/S shall not be held liable for any damage resulting from handling or contact with the above product.

Product disclaimer

ChemoMetec A/S reserves the right to introduce changes in the product to incorporate new technology. This application note is subject to change without notice.

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