

## Application note No. 3014. Rev. 1.2

# NucleoCounter® NC-3000™

## Viability and Cell Count using the Via1-Cassette™ - Purified Leukocytes

### Product description

The NucleoCounter® NC-3000™ system enables the user to perform automated cell counting and measure viability of purified leukocytes.

### Application

The Via1-Cassette™ used together with the NucleoCounter® NC-3000™ facilitates determination of viability and concentration of purified leukocytes by measuring cell counts (total and non-viable) per volume. The presence of more than  $5 \cdot 10^6$  erythrocytes /ml may quench fluorescence light due to the hemoglobin, resulting in a reduced cell count. In this case use the "Viability and Cell Count - Blood Assay".

### Introduction

In order to determine viability and cell concentration, a sample containing leukocytes is drawn into the Via1-Cassette™. The inside of the Via1-Cassette™ is coated with two different dyes, Acridine Orange staining the entire population of cells and DAPI staining the non-viable cells, respectively. The volume of each Via1-Cassette™ has been calibrated to give a high precision of the resulting count.

The Via1-Cassette™ is placed in the NucleoCounter® NC-3000™ where cell concentration and viability are determined.

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## Procedures

### Materials needed

- Leukocyte sample to be counted
  - Via1-Cassette™
1. The cell suspension is mixed to obtain a homogenous suspension. Draw a cell sample by inserting the tip of the Via1-Cassette™ into the cell suspension and pressing the piston.
  2. Immediately place the loaded Via1-Cassette™ on the tray of the NucleoCounter® NC-3000™, select the "**Viability and Cell Count – Purified Leukocytes Assay**" and sample unit **Via1-Cassette™** and press RUN.

After approximately 1 minute the viability (in percent) and the concentrations (cells/ml) of all cells is displayed in the bottom right of the user interface. Extended results are available in the result tab. If the cell sample has been 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 sample.

### Note

To assure reliable results, it is recommended that the total cell concentration of the leukocyte suspension should be in the range of  $5 \cdot 10^4$  cells/ml to  $5 \cdot 10^6$  cells/ml. 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 growth media or PBS. The resuspended cell sample is then treated as described above. By inserting the value for the dilution volume in the dilution field in the user interface the returned cell concentration is for the original cell sample.

If the total cell concentration is above  $5 \cdot 10^6$  cells/ml, the cell suspension can be diluted with growth media or PBS to achieve the desired concentration. The diluted 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 in the user interface the returned cell concentration is for the original cell sample.

## Viability

The viability is calculated as follows:

$$\% \text{ viability} = \frac{C_t - C_{nv}}{C_t} \cdot 100\%$$

**% viability**

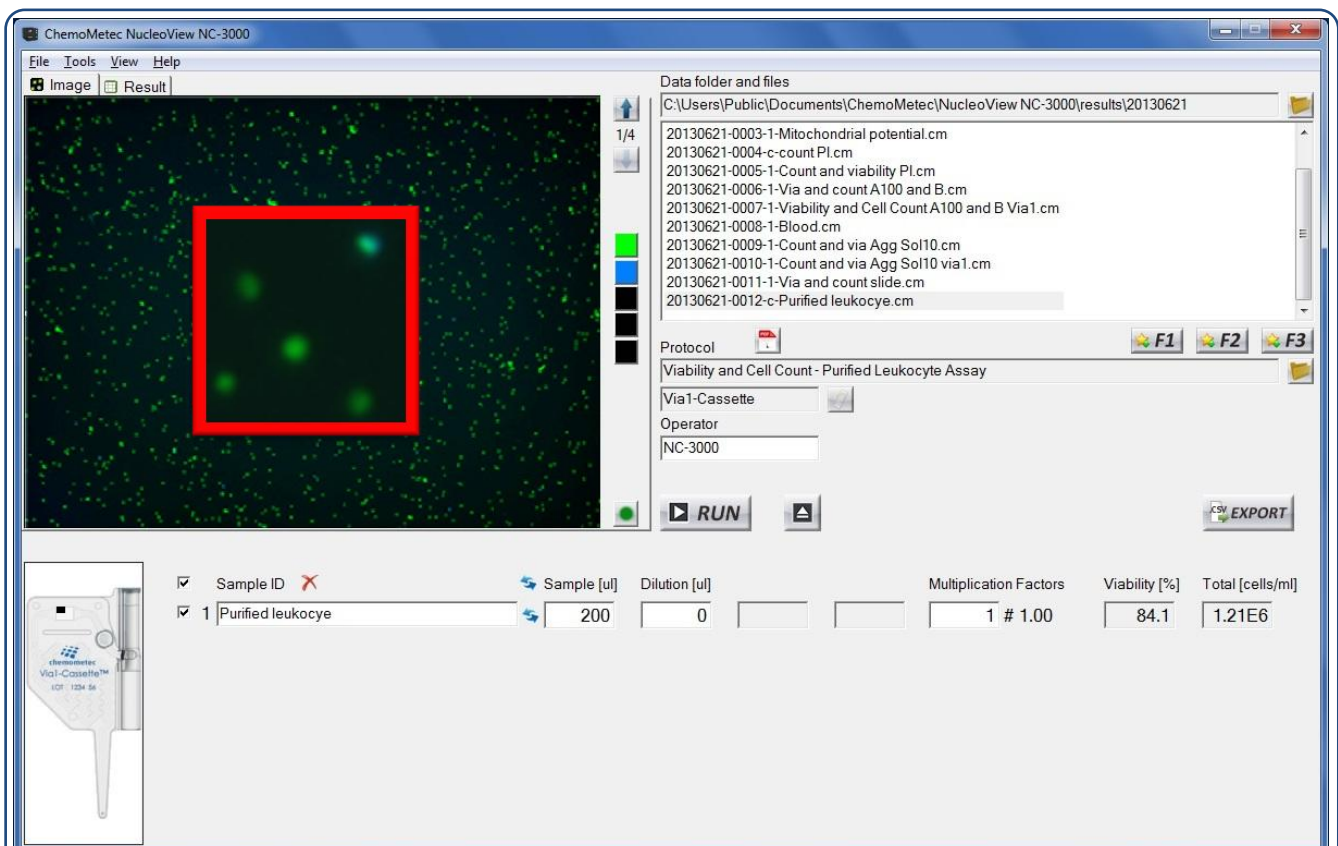
The percentage of viable cells in the sample

**C<sub>t</sub>**

The total concentration of cells

**C<sub>nv</sub>**

The concentration of non-viable cells



The screenshot shows the ChemoMetec NucleoView NC-3000 software interface. The main window displays a fluorescence microscopy image of leukocytes, with a red box highlighting a region of interest. The interface includes a menu bar (File, Tools, View, Help), a toolbar (Image, Result), and a data folder and files list. The protocol is set to "Viability and Cell Count - Purified Leukocyte Assay". The operator is identified as "NC-3000". A data table at the bottom shows the following results:

Sample ID	Sample [ul]	Dilution [ul]	Multiplication Factors	Viability [%]	Total [cells/ml]
1 Purified leukocyte	200	0	1 # 1.00	84.1	1.21E6

Determination of count and viability of leukocytes. The leukocyte suspension was loaded into a Via1-Cassette™ and analyzed using Viability and Cell Count – Purified Leukocyte protocol. All leukocytes are stained with acridine orange and appear green while non-viable leukocytes are stained with DAPI that appear blue. An insert shows a close up of a part of the image.

## 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 Raw Data". 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.

#### **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-3000™ 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-3000™ system is FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE. The results presented by the NucleoCounter® NC-3000™ system depend on correct use of the reagents, NC-slide and the NucleoCounter® NC-3000™ instrument and might depend on the type of cells being analyzed. Refer to the NucleoCounter® NC-3000™ 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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