

Application note No. 3034. Rev. 1.0

NucleoCounter® NC-3000™

Viability and Cell Count Method 2 using the Via1-Cassette™ - Mammalian cells

Product description

The NucleoCounter® NC-3000™ system enables the user to perform automated cell counting and analyses of a broad range of mammalian cells.

Application

The Via1-Cassette™ used together with the NucleoCounter® NC-3000™ facilitates determination of viability and concentration of cell suspensions by measuring cell counts (total and non-viable) per volume in one simple step. Blood and/or hemoglobin present in cell samples quench fluorescence light resulting in reduced cell counts. Use the viability and cell count application for blood or purified leukocytes to analyze samples with blood components.

Introduction

The Method 2 analysis is optimized for detection of dead cells with dim signals. Note that using the method 2 will increase the possibility of counting foreign objects and bubbles as cells. Furthermore, be aware that cell remnants from one cell can be counted at multiple cells. The technology used in method 2 does not allow for analysis of the cell area, diameter and the aggregation of cells.

In order to determine viability and cell concentration, a sample containing cells in suspension 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.

Procedures

If the cell line to be investigated is adherent or semi-adherent, then start by getting all cells into suspension using the preferred method of your laboratory (e.g. trypsin/EDTA treatment). Although NucleoCounter® NC-3000™ is able to count aggregated cells the accuracy is higher for single cell suspensions. If higher precision in cell count is needed when analysing a cell line with **aggregation** we recommend using the disaggregation and counting application.

Materials needed

- Cells 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 – Method 2 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 are displayed in the result fields. Moreover, in the result tab extended results are available showing the cell concentration of non-viable cells and information about cell aggregation (“clumpiness”). 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 cell 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.

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.

If the cell sample has been diluted or concentrated and the user has entered the volumes or dilution factor into the user interface, this dilution factor has also been taken into account and the returned cell concentration is for the original cell sample.

Viability

The viability is calculated by the NucleoView NC-3000™ software as follows:

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

% viability

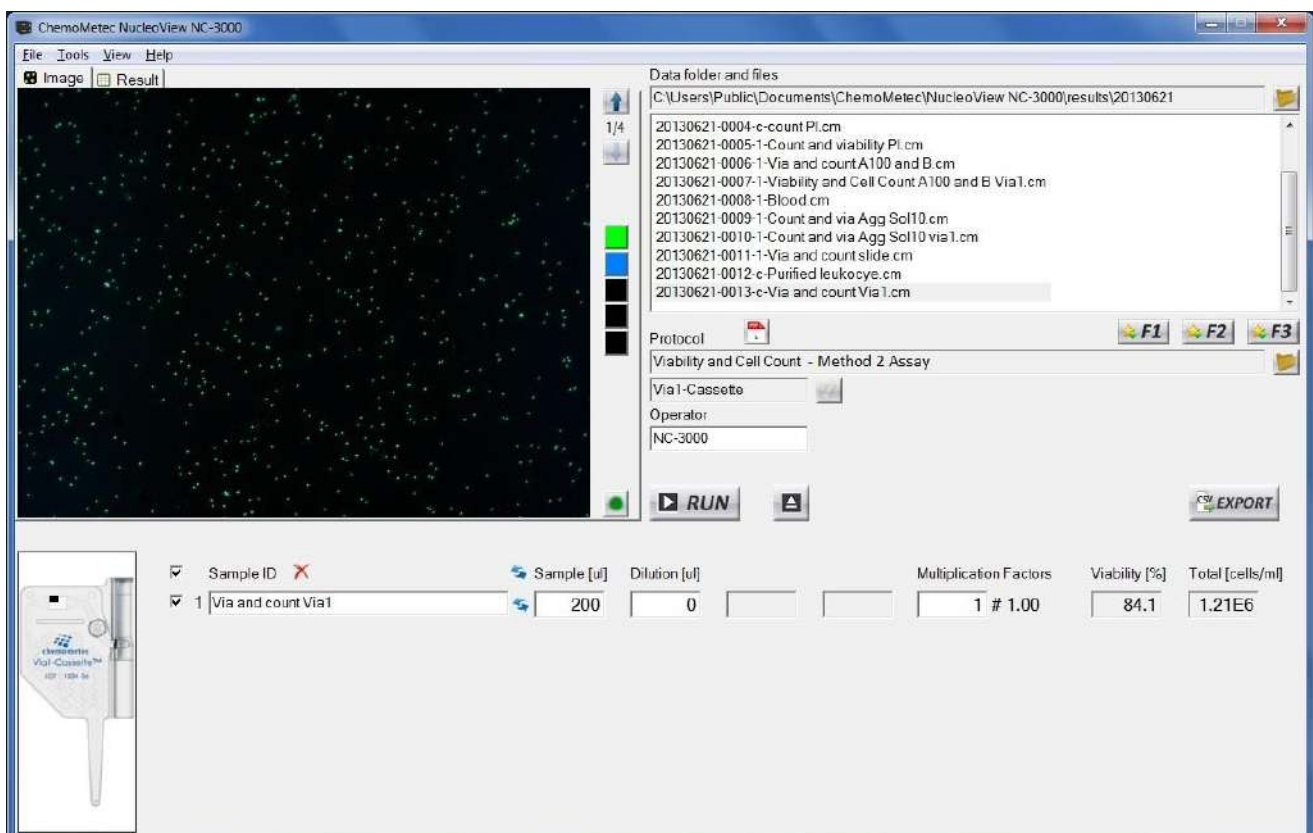
The percentage of viable cells in the cell suspension

C_t

The total concentration of cells

C_{nv}

The concentration of non-viable cells



Sample ID	Sample [ul]	Dilution [ul]	Multiplication Factors	Viability [%]	Total [cells/ml]
1 Via and count Via1	200	0	1 # 1.00	84.1	1.21E6

Determination of count and viability of WeHi cells. The cells were harvested, loaded into a Via1-Cassette™ and analyzed using the Viability and Cell Count – Method 2 Assay. The image acquired using NC-3000™ is shown (left). The total cell population is stained with acridine orange and appears green while non-viable cells are stained with DAPI that appear blue.

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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