

**NucleoCounter® NC-3000™ system**  
**Application note No. 011. Rev. 1.1**

## Cell concentration and viability (Via1-Cassette™)

### Product description

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

### Application

The Via1-Cassette™ used together with the NucleoCounter® NC-3000™ facilitates the determination of the viability and concentration of cell suspensions from a wide range of cells by measuring cell counts (total and non-viable) per volume in one simple step.

### Introduction

In order to determine viability and cell concentration, 50 µL sample containing cells in suspension is drawn into the Via1-Cassette™. The inside of the Via1-Cassette™ is coated with two different dyes, staining the entire population of cells and the non-viable cells, respectively.

The Via1-Cassette™ is placed in the NucleoCounter® NC-3000™ where the 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.

### Materials needed

- Cells to be counted
  - Via1-Cassette™
1. Draw a sample of cells in suspension 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 protocol "Viability and Cell concentration protocol" and press RUN.
  3. After approximately 45 seconds the viability (in percent) and the concentrations (cells/mL) of all cells and non-viable cells is displayed in the result box on the computer.

### 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  $1 \cdot 10^7$  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 or PBS media. The resuspended cell sample is then treated as described above.

If the total cell concentration is above  $1 \cdot 10^7$  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.

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#### Viability

The viability is calculated by the NucleoCounter software as follows:

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

<b>% viability</b>	The percentage of viable cells in the cell suspension
<b>C<sub>t</sub></b>	The total concentration of cells
<b>C<sub>nv</sub></b>	The concentration of non-viable cells

#### 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 analysed. 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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