Application note No. 0258

NucleoCounter® NC-250™

Mammalian Cells - Viability and Cell Count using NC-Slides

Product description

The NucleoCounter® NC-250™ system enables the user to perform automated cell counting and analyses of a broad range of eukaryotic cells.

Application

The NC-Slides and **Solution 18** used together with the NucleoCounter® NC-250™ facilitates determination of viability and concentration of cell suspensions by measuring cell counts (total and non-viable) per volume. The NC-Slide A2™ enables measurements of 2 cell samples at the same time with a high degree of precision, whereas the NC-Slide A8™ enables measurements of up to 8 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. An NC-slide with either two or eight samples is analyzed in approximately 3 minutes or 90 seconds, respectively. 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 to analyze samples containing red blood cells.

Introduction

In order to determine viability and cell concentration, a sample containing cells in suspension is mixed with **Solution 18** and loaded into a NC-Slide. **Solution 18** contains two different dyes, staining the entire population of cells and the non-viable cells, 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 a NC-Slide is 100 µm, with 90 % of all chambers being in the range from 90-110 µm.

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-250™ 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 counting application for aggregating cells.

Materials needed

- Cells to be counted
- NC-Slide A2™ or NC-Slide A8™
- **Solution 18**

1. The cell suspension is mixed to obtain a homogenous suspension. Pipette a representative cell sample from the cell suspension into a microcentrifuge tube. Add one volume of **Solution 18** into 20 volumes of the cell suspension. E.g., if the volume of the cell suspension is 100 µl then add 5 µl **Solution 18**. Mix by pipetting.
2. 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 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 cells and non-viable cells is displayed. The cell concentrations have been compensated for the dilution caused by the addition of **Solution 18**. 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.

Notes

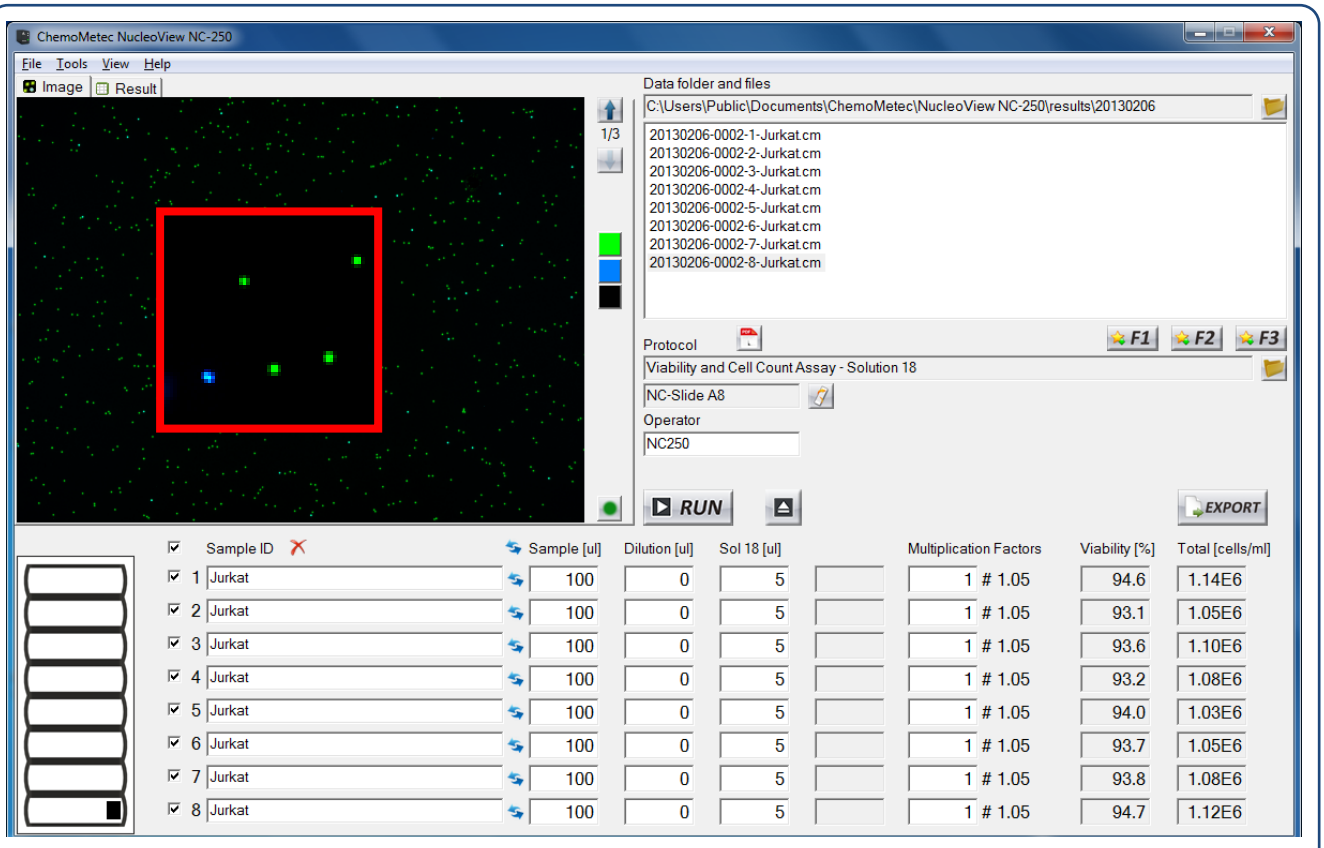
To assure reliable results, it is recommended that the cell concentration of the counted cell suspension should be in the range of $5 \cdot 10^4$ cells/ml to $5 \cdot 10^6$ cells/ml. If the cell concentration is above $5 \cdot 10^6$ cells/ml, the cell suspension can be diluted with growth media or PBS 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 growth media or 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



Determination of count and viability of Jurkat cells. The cells were stained with **Solution 18**, loaded into an NC-slide™ and analyzed using the Viability and Cell Count protocol. The total cell population is stained with acridine orange and appears green while non-viable cells are stained with DAPI that 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 adapt the gate(s) to cover the cell population (do not include debris and very large objects) using the Protocol Adaptation Wizard and save the changes to a new protocol. Note that the user is responsible for defining appropriate gating of the particular cell line.

Unexpectedly high viability:

Cells that have been dead for a long period of time contain degraded nucleic acid. The dyes used in this assay primarily binds to nucleic acid and consequently the fluorescent signals from dead cells may be exceptionally dim. Very dim signals can be observed in cells that, for example, have been growing for a long period of time in a bioreactor and in some suspension cell lines. Dim signals can be detected by enabling method 2 in the Protocol Adaptation Wizard. Note that method 2 increases the possibility of counting foreign objects and bubbles as cells. Furthermore, be aware that when cells die they will at some point break up to smaller parts and that these cell remnants can be counted at multiple cells. The procedure used in method 2 does not allow for analysis of the cell area, diameter and the aggregation of 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-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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