

Application note No. 3012. Rev. 1.4

NucleoCounter® NC-3000™**Viability and Cell Count using NC-Slides™ - Insect cells****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 NC-Slides™ and **Solution 13** 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. 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-Slides™ 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.

Introduction

In order to determine viability and cell concentration, a sample containing cells in suspension is mixed with **Solution 13** and loaded into a NC-Slide™. **Solution 13** contains two different dyes, Acridine Orange staining the entire population of cells and DAPI staining the non-viable cells, respectively. After loading the NC-Slide™ it is placed in the NucleoCounter® NC-3000™ 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. If higher precision in cell count is needed we recommend to use the volume calibrated Via1-Cassette.

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
 - **NC-Slide A2™** or **NC-Slide A8™**
 - **Solution 13**
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 13** into 19 volumes of the cell suspension. E.g., if the volume of the cell suspension is 190 µl then add 10 µl **Solution 13**. 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-3000™ and select "**Viability and Cell Count Assay (Insect Cells)**" and sample unit **NC-Slide A2™** or **NC-Slide A8™** and press RUN.

After analysis the viability (in percent) and the total cell concentrations (cells/ml) are presented in the bottom right result box. Extended results are presented in the results tab page. The cell concentrations have been compensated for the 5% dilution caused by the addition of **Solution 13**. If the cell sample has been further 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.

Notes

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.

Viability

The viability is calculated as follows:

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

% viability

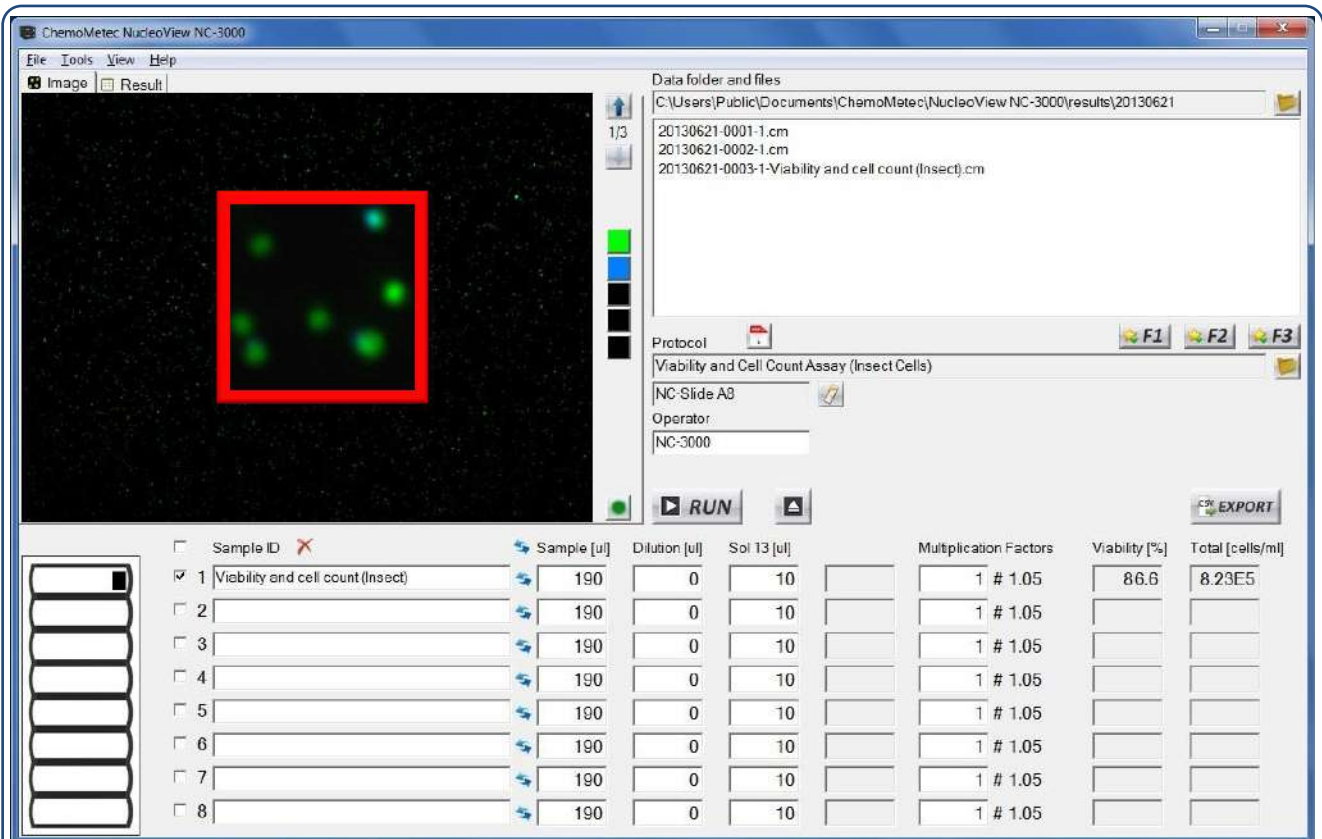
The percentage of viable cells in the cell suspension

C_t

The total concentration of cells

C_{nv}

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 cells, 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 table at the bottom. The data table has the following columns: Sample ID, Sample [ul], Dilution [ul], Sol 13 [ul], Multiplication Factors, Viability [%], and Total [cells/ml]. The first row is checked and shows a viability of 86.6% and a total cell count of 8.23E5.

Sample ID	Sample [ul]	Dilution [ul]	Sol 13 [ul]	Multiplication Factors	Viability [%]	Total [cells/ml]
1	Viability and cell count (Insect)	190	0	10	86.6	8.23E5
2		190	0	10		
3		190	0	10		
4		190	0	10		
5		190	0	10		
6		190	0	10		
7		190	0	10		
8		190	0	10		

Determination of count and viability of S2 cells. The cells were harvested, stained with **Solution 13**, loaded into an NC-slide™ and analyzed using the Viability and Cell Count Assay (Insect Cells). 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.

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.

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 cover slip. 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.

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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