Application note No. 3018. Rev. 1.2

NucleoCounter® NC-3000™

Viability and Cell Count using the Via1-Cassette™ - Aggregated Mammalian Cells

Product description

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

Application

The Via1-Cassette™ and **Solution 10** used together with the NucleoCounter® NC-3000™ can be used for determination of the viability and cell concentration of aggregating cells. Treatment of cell samples with **Solution 10** facilitates disaggregation of cell aggregates resulting in single cell suspensions. At the same time **Solution 10** also enables staining of all cells with DAPI.

Introduction

In order to determine the cell concentration, a sample containing cells in suspension is diluted with **Solution 10** (lysis buffer) and drawn into the Via1-Cassette™. The inside of the Via1-Cassette™ is coated with DAPI, which after lysis with **Solution 10** stains all cell nuclei in the sample. When the cell sample is not treated with **Solution 10** the dye only stains the non-viable cells. 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 is 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).

Materials needed

- Cells to be counted
- **Solution 10**
- Two Via1-Cassettes™

1. The first step is to determine the total cell concentration of a cell sample that is lysed with **Solution 10**.
    a. The cell suspension is mixed to obtain a homogenous suspension and a sample is mixed 1:1 with **Solution 10**. E.g., to 100 µl of cell suspension add 100 µl of **Solution 10**. Mix by pipetting. Draw a cell sample by inserting the tip of the first Via1-Cassette™ into the diluted cell suspension and pressing the piston.
    b. Select the "**Viability and Cell Count - Aggregated Cells Assay**" and sample unit "**Via1-Cassette™"** and press RUN. Click "**OK**" when the loaded Via1-Cassette™ containing the sample diluted 1:1 with **Solution 10** is in place on the tray of the NucleoCounter® NC-3000™.
2. The second step is to determine the concentration of non-viable cells of an undiluted cell sample.
    a. The undiluted cell suspension (**without Solution 10** treatment) is mixed again to obtain a homogenous suspension. Draw a cell sample by inserting the tip of the second Via1-Cassette™ into the cell suspension and pressing the piston.
    b. When the message box requests it, replace the first Via1-Cassette™ with the second Via1-Cassette™ loaded with the undiluted cell suspension and click "**OK**".

After approximately 2 minutes the cell concentration (cells/ml) of the total cell count is displayed in the result fields, together with the viability. Extended results are available in the result tab. The displayed cell concentration of the total cell count has been compensated for the two time dilution caused by the addition of **Solution 10**. 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.

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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.

Viability

The viability is calculated as follows:

$$\% \text{ viability} = \frac{Ct - Cnv}{Ct} \cdot 100\%$$

% viability	The percentage of viable cells in the cell suspension
$\mathbf{C_t}$	The total concentration of cells
$\mathbf{C_{nv}}$	The concentration of non-viable cells

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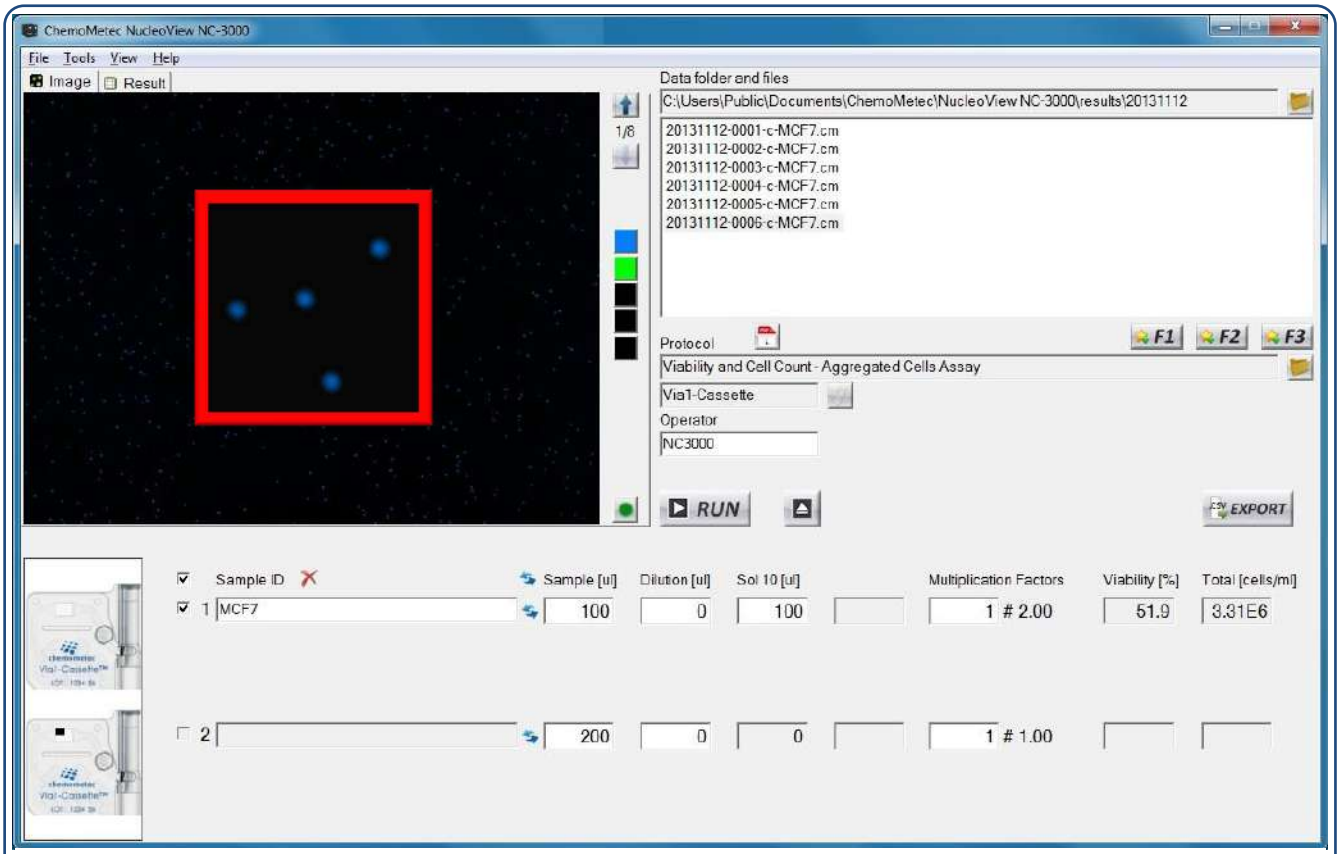

Determination of cell concentration of aggregated MCF7 cells. The cells for the first cassette were disaggregated by adding **Solution 10** and the cells for the second cassette were untreated. The two samples were loaded into Via1-Cassettes™ and analyzed using the Viability and Cell Count - Aggregated Cells Assay. The cell populations are stained with DAPI and 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.

Warning that the cell concentration of non viable cells is higher than the total cell concentration:

Make sure the problem is not due to interchanged samples of the total count sample treated with **Solution 10** and the untreated non-viable count sample. If the samples have not been interchanged the continued warning can be due to a very high frequency of non-viable cells in the sample.

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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