Application note No. 3030. Rev. 1.1

NucleoCounter® NC-3000™

Counting Aggregated Cells using the Via1-Cassette™ with Reagent A100 and B

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 **Reagent A100** and **B** used together with the NucleoCounter® NC-3000™ facilitate determination of the cell concentration of aggregating cell lines. Treatment of cell samples with **Reagent A100** facilitates disaggregation of cell aggregates resulting in single cell suspensions. **Reagent A100** also enables staining of all cells with DAPI. **Reagent B** stabilizes the nuclei for the analysis.

Introduction

In order to determine the cell concentration, a sample containing cells in suspension is diluted with **Reagent A100** (lysis buffer) followed by stabilization with **Reagent B** and drawn into the Via1-Cassette™. The inside of the Via1-Cassette™ is coated with DAPI, which after lysis with **Reagent A100** stains all cell nuclei in the sample. 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
- **Reagent A100**
- **Reagent B**
- Via1-Cassette™

1. Mix the cell suspension to obtain a homogenous suspension and add one volume of **Reagent A100**. For example, to 100 µl of cell suspension add 100 µl of **Reagent A100**. Mix by pipetting.
2. Add 1 volume of **Reagent B** to the mixture of cell suspension and **Reagent A100**e.g. to 200 µl of the mixture of cell suspension and **Reagent A100** add 100 µl of **Reagent B**. Mix by pipetting.
3. Draw a cell sample into the Via1-Cassette™ by inserting the tip into the suspension and pressing the piston.
4. Immediately place the loaded Via1-Cassette™ on the tray of the NucleoCounter® NC-3000™, select the "**Count of Aggregated Cells – A100 and B Assay**" and sample unit **Via1-Cassette** and press RUN.

After approximately 1 minute the cell concentration (cells/ml) is displayed in the result field. The displayed cell concentration has been compensated for the three-fold dilution caused by **Reagent A100** and **B**. If the sample has been further diluted and the user has entered the volumes or dilution factor into the user interface, the dilution factor has also been taken into account and the cell concentration given 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.

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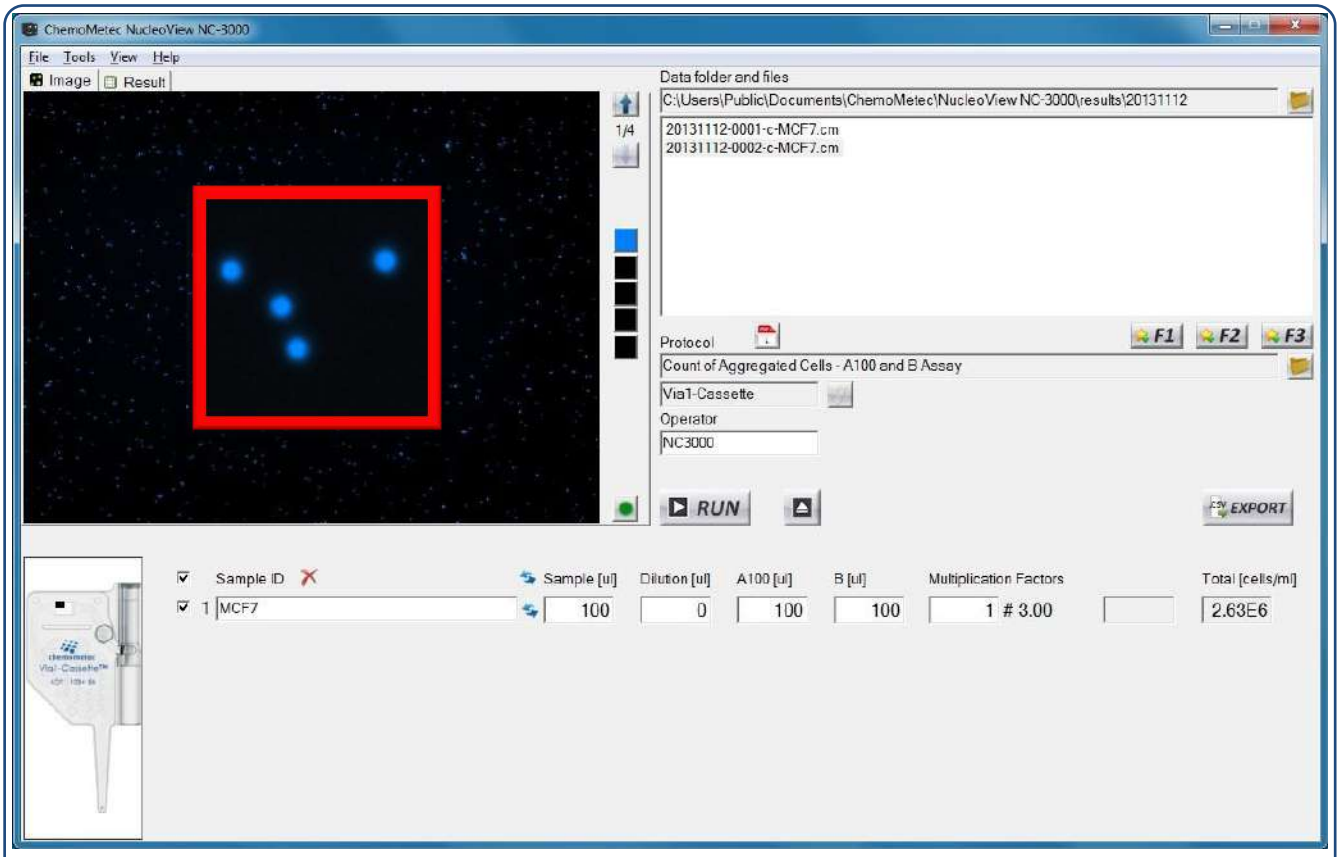
If the total cell concentration is above $5 \cdot 10^6$ cells/ml, the cell suspension can be diluted with growth media or PBS to achieve the desired concentration. The diluted cell sample is then treated as described in the procedure.



Determination of cell concentration of aggregated MCF7 cells. The cells were disaggregated by adding **Reagent A100** followed by stabilization with **Reagent B**. The sample was loaded into a Via1-Cassete™ and analyzed using the Count of Aggregated Cells – A100 and B Assay. The total cell population is stained with DAPI and appears blue. An insert shows a close up of a part of the image.

Trouble shooting

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.

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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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