Application note No. 3027. Rev. 1.1

NucleoCounter® NC-3000™

Viability and Cell Count using NC-slides A2™ with Reagent A100 and B

Product description

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

Application

The NC-Slide A2™, **Reagent A100**, **Reagent B** and **Solution 12** used together with the NucleoCounter® NC-3000™ facilitates determination of the viability and cell concentration of aggregating cells. Treatment of cells with **Reagent A100** facilitates disaggregation of cell aggregates resulting in single cell suspensions. At the same time **Solution 12** enables staining of all cells with DAPI. **Reagent B** stabilizes the nuclei for the analysis. One sample is analyzed in approximately 90 seconds.

Introduction

In order to determine the total cell concentration, a sample containing cells in suspension is mixed with **Reagent A100** (lysis buffer) and **Solution 12** (staining solution). After stabilization with **Reagent B** the sample is loaded into a NC-Slide A2™. **Solution 12** contains DAPI, which stains all cell nuclei after lysis with **Reagent A100**. The nuclei are then stabilized with **Reagent B**. In order to also determine the non-viable cell concentration for calculating the viability, the cell sample is mixed with **Solution 12** containing DAPI, which stains the non-viable cells. After loading the NC-Slide A2™ it is placed in the NucleoCounter® NC-3000™ where viability and cell concentration is determined.
The nominal depth of the chambers in the NC-Slide A2™ 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 releasing all cells into suspension using the method preferred in your laboratory (e.g. trypsin/EDTA treatment).

Materials needed

- Cells to be counted
- NC-Slide A2™
- **Reagent A100** (Lysis buffer)
- **Reagent B** (Stabilizing buffer)
- **Solution 12** (500 µg/ml DAPI)

Two cell samples are required to perform a viability and cell count determination on aggregated cell suspensions. The first sample is treated with **Reagent A100** and **Solution 12** followed by treatment with **Reagent B** and determines the total cell concentration while the second cell sample is just treated with **Solution 12** to determine the concentration of the non-viable cells.

1. Sample 1: Total cell count
    a. Add 1 volume of **Solution 12** into 99 volumes of **Reagent A100** e.g., add 10 µl of **Solution 12** to 990 µl **Reagent A100**. Note: do not store this mixture, but prepare a new each time the assay is performed.
    b. The original cell suspension is mixed to obtain a homogenous suspension. Pipette a representative cell sample from the cell suspension into a microcentrifuge tube (e.g. 100 µl).
    c. Add 1 volume of the mixture of **Solution 12** and **Reagent A100** to the microcentrifuge tube with the cell sample e.g., if the volume of the cell sample is 100 µl then add 100 µl of the mixture of **Solution 12** and **Reagent A100**. Mix by pipetting.

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d. Add one volume of **Reagent B** to the mixture of cell suspension, **Reagent A100** and **Solution 12** e.g. to 200 µl of the mixture of cell suspension, **Reagent A100** and **Solution 12** add 100 µl of **Reagent B**. Mix by pipetting.
e. Load ~30 µl of the cell sample for the total cell count into chamber 1 of the slide.

2. Sample 2: Non viable cell count:
    a. Mix the original cell suspension again to obtain a homogenous suspension. Pipette a representative cell sample from the cell suspension into a microcentrifuge tube (e.g. 199 µl).
    b. Add 1 volume of **Solution 12** to 199 volumes of cells sample in the microcentrifuge tube e.g., if the volume of the cell sample is 199 µl then add 1 µl of **Solution 12**. Mix by pipetting.
    c. Load ~30 µl of the sample for the non-viable cell count into chamber 2 of the slide.
3. Place the loaded slide on the tray of the NucleoCounter® NC-3000™ and select "**Viability and Cell Count – A100 and B Assay**" and sample unit **NC-Slide A2** and press RUN.

After approximately 90 seconds, the cell concentrations (cells/ml) of the total cell count and the count of non-viable cells are displayed in the result fields, together with the viability. The displayed total cell concentration has been compensated for the three-fold dilution caused by addition of **Reagent A100**, **Solution 12** and **Reagent B**. Similarly, the cell concentration for the non-viable cells has been compensated for the dilution caused by the addition of **Solution 12**. 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 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.

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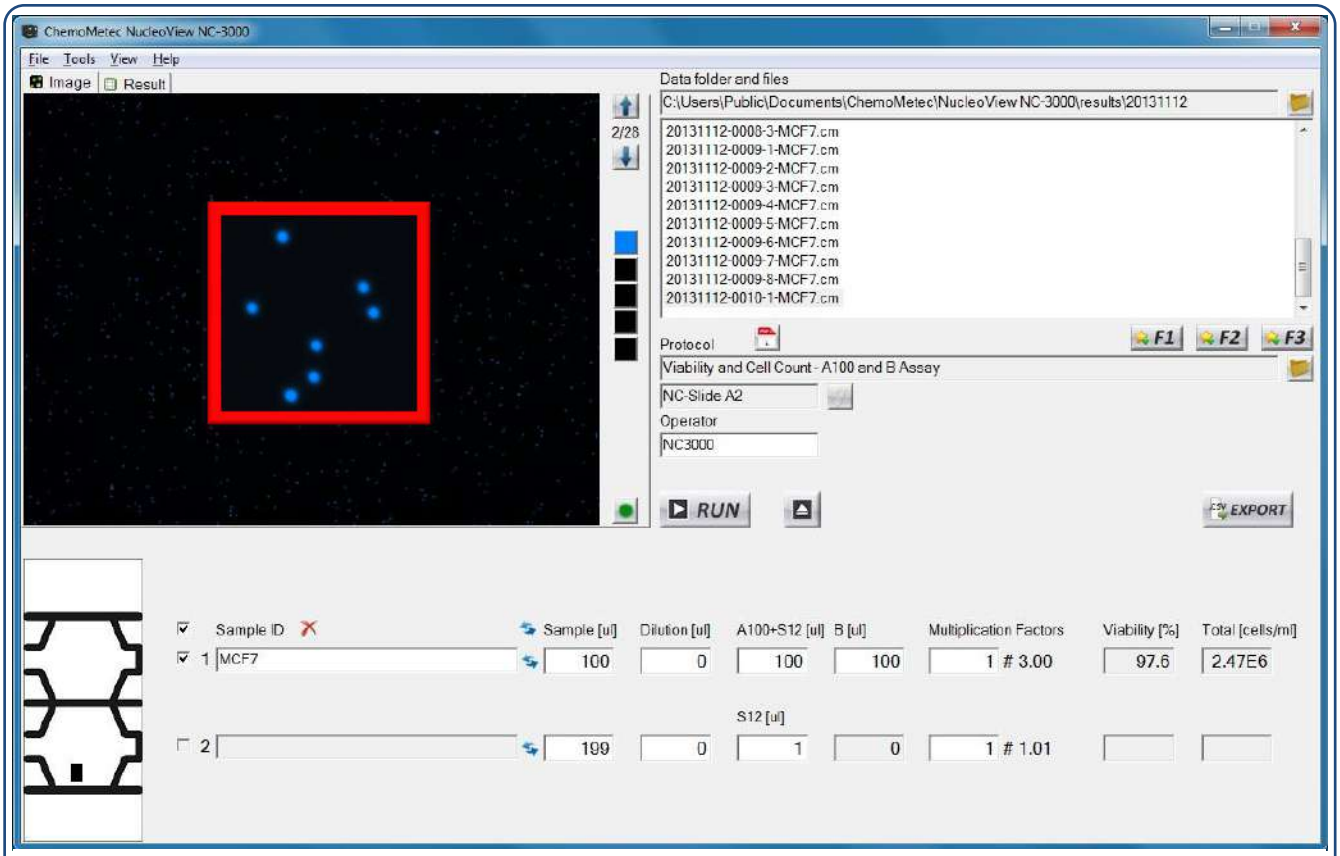
Viability

The viability is calculated as follows:

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

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



Determination of viability and cell concentration of MCF7 cells. The cells for the first chamber were disaggregated and stained by adding a mixture of **Reagent A100** and **Solution 12**, followed by stabilization with **Reagent B**. The cells for the second chamber were only treated with **Solution 12**. The cell populations are stained with DAPI and appear blue. An insert shows a close up of a part of the image.

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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 coverslip. 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.

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 a mixture of **Reagent A100**, **Solution 12** and **Reagent B** and the non-viable count sample only treated with **Solution 12**. 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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