Application note No. 3032

NucleoCounter® NC-3000™

Count and Viability of PI Stained Mammalian 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 PI-Cassette™ is used together with **Reagent A100** and **B** and analyzed using the NucleoCounter® NC-3000™. Thereby it facilitates the determination of cell concentration even if the cell type investigated exhibit a very aggregating phenotype and the cell concentration cannot accurately be determined using the Via1-Cassette™.

Introduction

Propidium iodide (PI) is immobilized inside the PI-Cassette™ and has the ability to stain DNA of non-viable cells. PI enters non-viable cells, as their plasma membrane is permeable. In order to measure the total concentration of cells the plasma membranes of all cells in the sample must be disrupted to render all nuclei susceptible to staining with PI. The disruption is achieved by treatment with a lysis buffer (**Reagent A100**) followed by a stabilizing buffer (**Reagent B**). After treating the cells with the **Reagent A100** and **B** the suspension is loaded into the PI-Cassette. Once inside the PI-Cassette™ the nuclei are stained with PI. The PI-Cassette™ is placed in the NucleoCounter® NC-3000™ where the 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
- Two PI-Cassettes™
- **Reagent A100** (Lysis buffer)
- **Reagent B** (Stabilizing buffer)

1. The first step is to determine the total cell concentration of a cell sample that is lysed with **Reagent A100** and stabilized with **Reagent B**.
    a. 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).
    b. Add 1 volume of **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 **Reagent A100**. Mix by pipetting.
    c. 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 **Reagent B**. Mix by pipetting.
    d. Draw the diluted cell suspension into a PI-Cassette™ by inserting the tip of the cassette into the cell suspension and press the piston.
    e. Select the "**Count and Viability of PI Stained Cells Assay**" and sample unit "**PI-Cassette™"** and press RUN. Click "**OK**" when the loaded Via1-Cassette™ containing the sample diluted 1:1: with Reagent A100 and B 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.

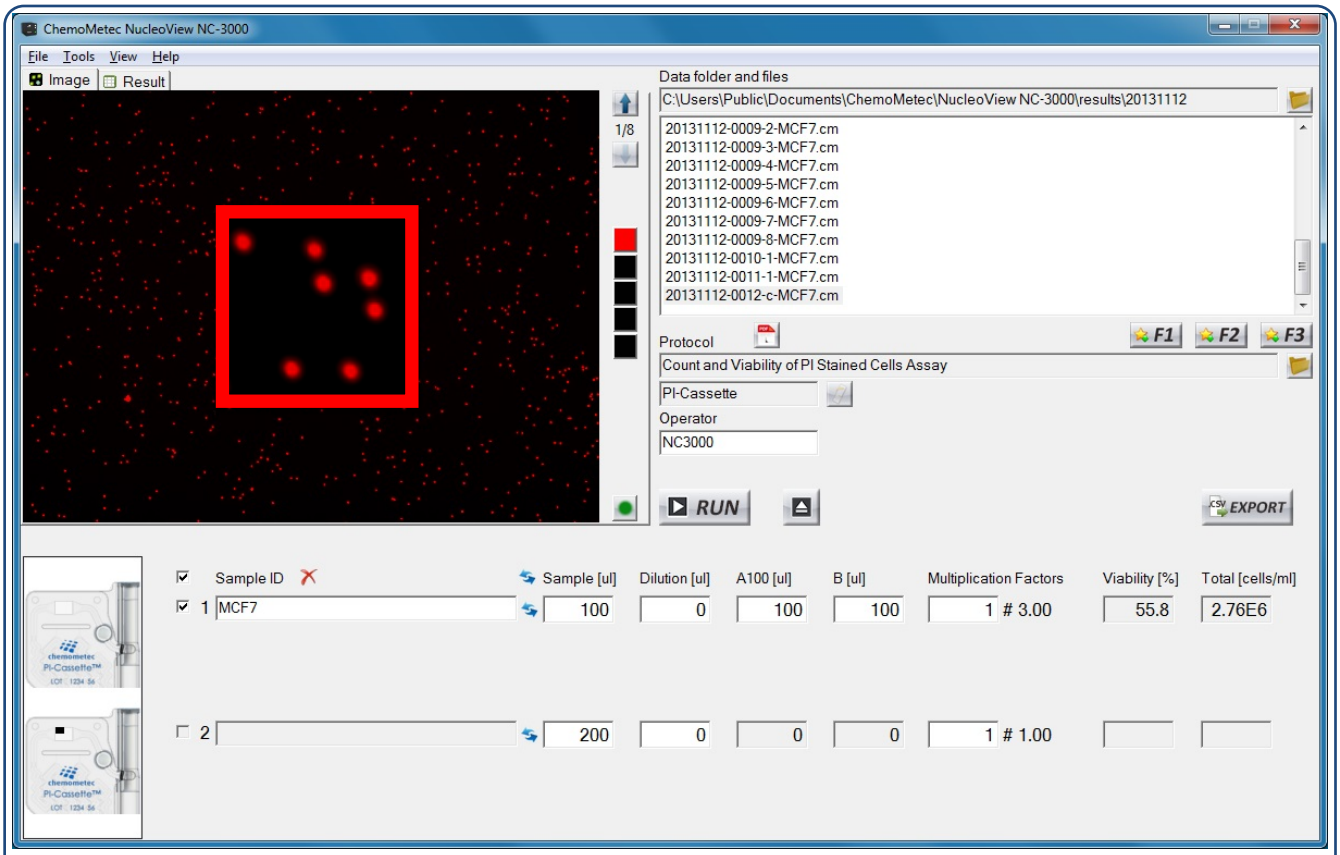
a. The undiluted cell suspension (**without Reagent A100** and **B** treatment) is mixed again to obtain a homogenous suspension. Draw a cell sample by inserting the tip of the second PI-Cassette™ into the cell suspension and pressing the piston.
b. When the message box requests it, replace the first PI-Cassette™ with the second PI-Cassette™ loaded with the undiluted cell suspension and click "**OK**".

After approximately 2 minutes the total cell concentration (cells/ml) is presented together with the viability in the result fields. Extended results are available in the result tab. The presented cell concentration of the total cell count has been compensated for the dilution caused by adding of **Reagent A100** and **Reagent 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.

If the total cell concentration is above $5{\cdot}10^6$ cells/ml, the cell suspension should 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 viability and cell concentration of aggregated MCF7 cells. The cells were harvested and the first sample was treated with **Reagent A100** and **B** was loaded into a PI-Cassette™ and the Count of PI stained Cells Assay was started. The second sample was loaded into a second PI-Cassette™ without treatment with any solution. 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 **Reagent A100** and **B** and the non-viable count sample without treatment. 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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