Application note No. 3031

NucleoCounter® NC-3000™

Count and Viability of PI Stained Yeast 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

This protocol for the NucleoCounter® NC-3000™ system enables the user to determine the cell density and viability of different yeast species (e.g. *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*). The protocol is suitable for use on yeast samples, which has a low degree of flocculation.

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 Y100**). After treating the cells with **Reagent Y100** 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. To count nonviable cells the cell suspension is loaded directly into a PI-Cassette™. Thereby only the nuclei of non-viable cells are stained when mixed with PI.
Having obtained the total concentration of cells and the concentration of non-viable cells, the viability can be calculated.

Procedure

Materials

- Cells to be counted*
- **Reagent Y100** (Lysis buffer)
- Two PI-Cassettes™

* provided by the user

Important notes:
Although NucleoCounter® NC-3000™ is able to count aggregated cells, the accuracy is higher for single cell suspensions.

1. The first step is to determine the total cell concentration of a cell sample that is lysed with **Reagent Y100**.
    a. Dispense 450 µl of **Reagent Y100** solution into a 1.5 ml microcentrifuge tube.

    b. Transfer 50 µl of the cell suspension to the reagent solution. Mix the solution well by vortexing (or by pipetting vigorously for a few seconds). The total volume of the mixture is then 500µl, giving a 10-fold dilution. Incubation times of up to 10 minutes may be necessary for different types of yeast.

    c. Draw a sample of cells in suspension by inserting the tip of the first PI-Cassette™ into the cell suspension and pressing the piston.

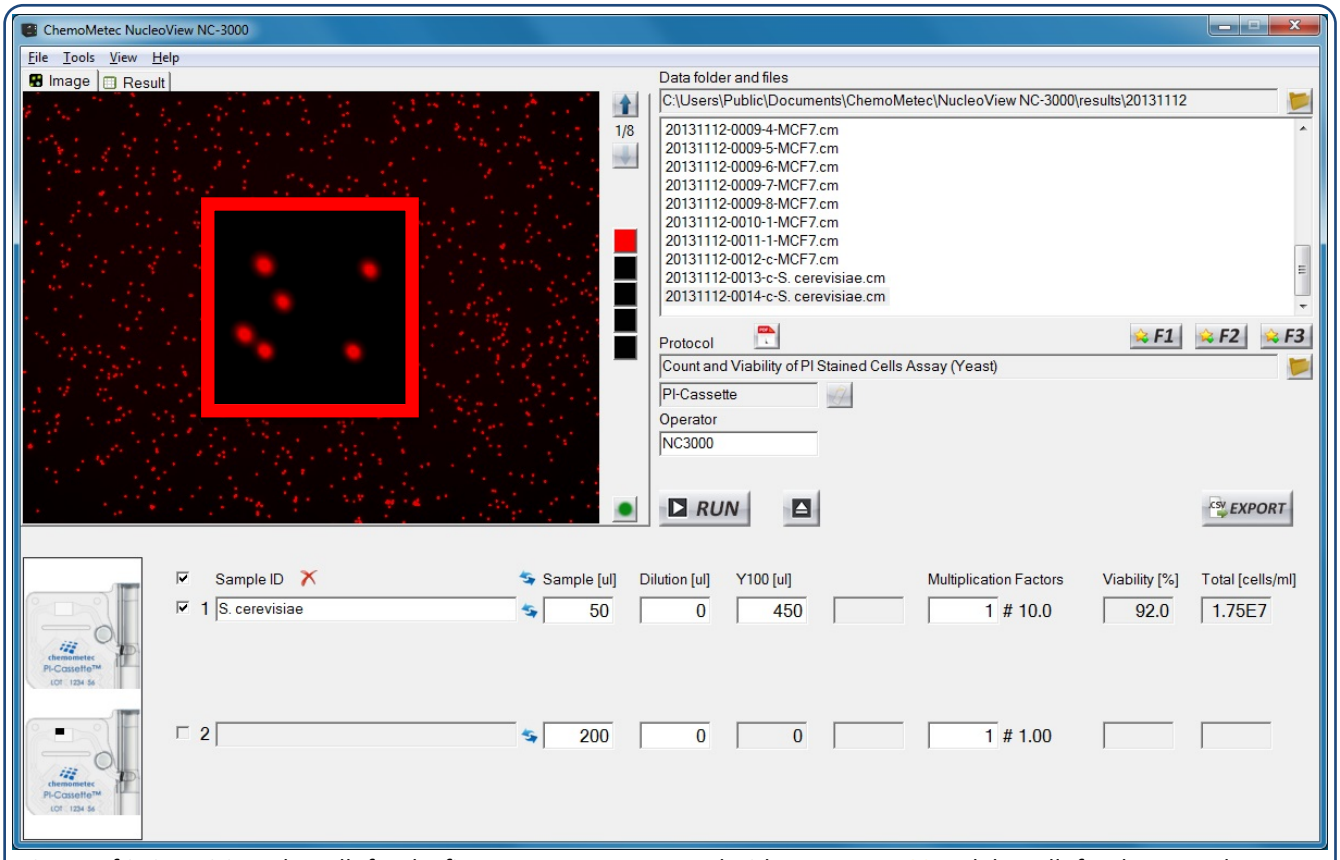
    d. Select the "**Count and Viability of PI Stained Cells Assay (Yeast)**" and sample unit "**PI-Cassette™"** and press RUN. Click "**OK**" when the loaded PI-Cassette™ containing the sample Treated with **Reagent Y100** is in place on the tray of the NucleoCounter® NC-3000™.

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 Y100** 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 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 ten time dilution caused by the addition of **Reagent Y100**. 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.

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 in growth media, PBS or H_2O. 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, PBS or H_2O to achieve the desired concentration. The diluted cell sample is then treated as described in the procedure.



Count of S. Cerevisiae. The cells for the first cassette were treated with **Reagent Y100** and the cells for the second cassette were untreated. The two samples were loaded into PI-Cassettes™ and analyzed using the Count of PI Stained Cells Assay (Yeast). 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 Y100** and the non-viable count untreated 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 and PI-cassettes 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 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.

Copyright