Application note No. 3009. Rev. 1.3

NucleoCounter® NC-3000™

Count of PI Stained Cells – Total Count of 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.

Procedure

Materials

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

* provided by the user

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

Total cell concentration

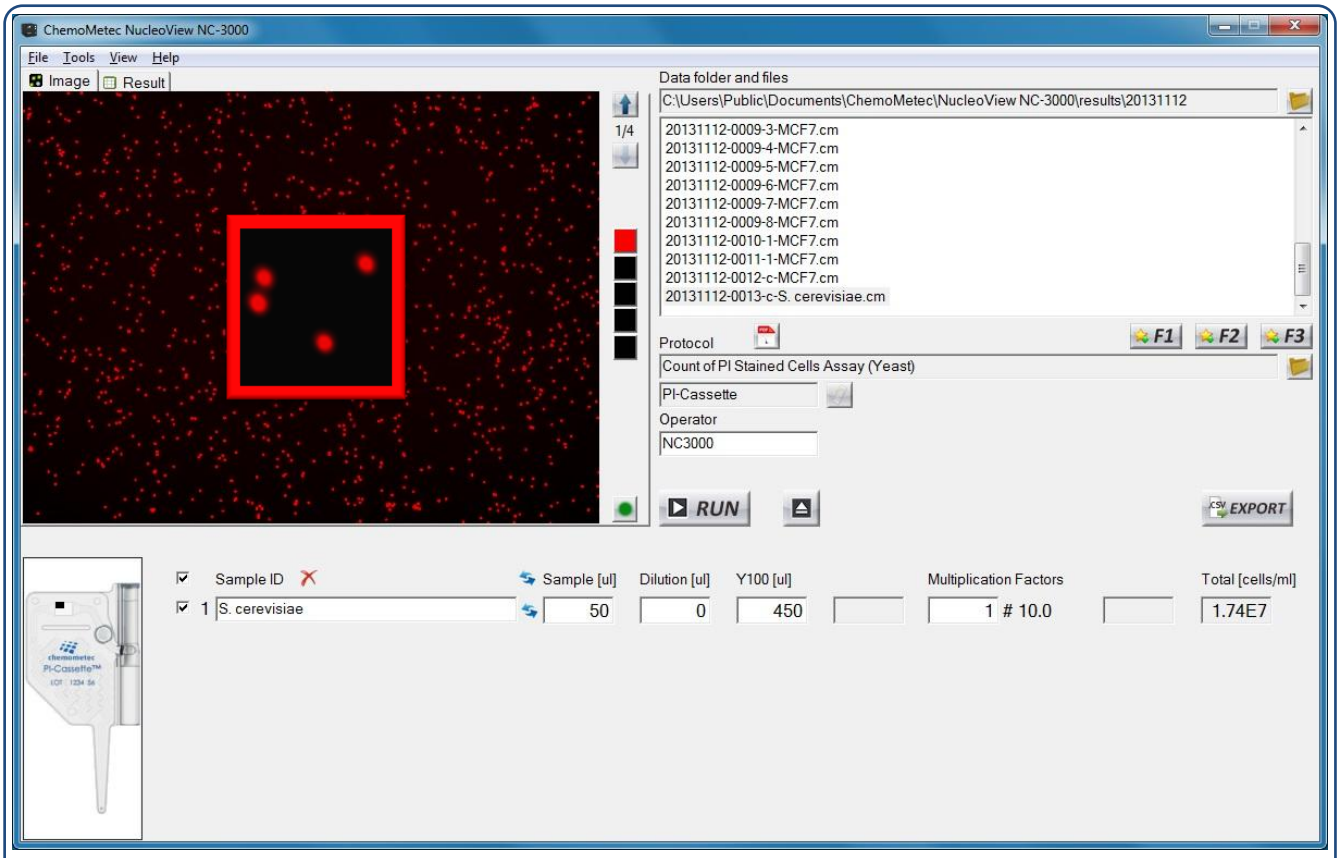
1. Dispense 450 µl of **Reagent Y100** solution into a 1.5 ml microfuge tube.
2. Transfer 50 µl of the cell suspension to the reagent solution. Mix the solution well by vortexing (or by pipetting vigorously for a few seconds). The total volume of the mixture is then 500µl, giving a 10-fold dilution. Incubation times of up to 10 minutes may be necessary for different types of yeast.
3. Draw a sample of cells in suspension by inserting the tip of the PI-Cassette™ into the cell suspension and pressing the piston.
4. Immediately place the loaded PI-Cassette™ in the NucleoCounter® NC-3000™ sample tray, select the protocol "**Count of PI Stained Cells Assay (Yeast)**" and press RUN.

After approximately 45 seconds the total cell concentration (cells/ml) is displayed in the result field. The cell concentrations have been compensated for the 10 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 factor has also been taken into account and the returned cell concentration is for the original cell sample.

Gydevang 43 • DK-3450 Allerod • Denmark • Phone: (+45) 48 13 10 20 • Fax: (+45) 48 13 10 21 • Support: contact@chemometec.com • Web: www.chemometec.com

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 were harvested, treated with **Reagent Y100**, loaded into a PI-Cassette™ 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.

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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.

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