Application Note No. 0303. Rev. 1.3

NucleoCounter® NC-202™

Count & Viability of Yeast Cells - Via2-Cassette™

Product description

The NucleoCounter® NC-202™ automated cell counter and NC-View™ software perform cell counting and viability analyses on a broad range of eukaryotic cells.

Application

This application note describes how to determine the cell concentration and viability of *Saccharomyces and Schizosaccharomyces* yeast cells using the Via2-Cassette™ and Reagent **Y200** (yeast permeabilization buffer). The Via2-Cassette™ provides a simple and robust method to determine cell concentration and viability with the NucleoCounter® NC-202™.

Introduction

Two Via2-Cassettes™ are required to determine cell count and viability for this application. To measure the total cell concentration, mix a sample of yeast cells in suspension, permeabilize with reagent **Y200**, and load into the first Via2-Cassette™, staining the total cell population with acridine orange (AO). The second Via2-Cassette™ determines the concentration of non-viable cell by staining an untreated sample with DAPI. Once loaded, place the Via2-Cassette™ in the NucleoCounter® NC-202™ and press RUN to acquire data. The NC-View™ software automatically analyses and presents cell concentration and viability for fast and easy data acquisition.

Procedure

Treat the yeast culture with reagent **Y200** to permeabilize yeast cells, to allow staining of the total cell population. Transfer a representative sample to a 1.5 ml microcentrifuge tube from which an aliquot can be drawn using the Via2-Cassette™.

<u>Materials needed</u>

- Yeast cell sample in suspension
- Reagent **Y200**
- Via2-Cassette™

<u>Procedure</u>

1. Determine the total cell concentration from a cell sample mixed 1:9 with reagent **Y200**:
   a. From a homogenous yeast cell sample, mix cells and reagent **Y200** at a 1:9-ratio, e.g. mix 50 µl of cell suspension and 450 µl reagent **Y200**
   b. Vortex or mix by vigorously pipetting the sample and load by inserting the tip of the first Via2-Cassette™ into the permeabilized cell suspension and pressing the piston
   c. Insert the Via2-Cassette™ into the NucleoCounter® NC-202™, select the 'Yeast' protocol, and press RUN

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2. Determine the non-viable cell concentration from an untreated cell sample:
   a. Mix the cell suspension (**without Y200** treatment) and with PBS at a 1:9-ratio, e.g. 50 µl of cell suspension and 450 µl PBS, to obtain a homogenous suspension. Insert the tip of the second Via2-Cassette™ into the suspension and load cell sample by pressing the piston
   b. When prompted by the NC-View™ message box, replace the first Via2-Cassette™ with the second, and select 'OK'

   NOTE: If the viability reading is not necessary, then select 'Cancel' after analysis of the first Via2-Cassette™, whereby only the total cell concentration will be provided.

Within one minute the viability and cell concentration of the sample are displayed. The available results are: Total (cells/ml), Live (cells/ml), Dead (cells/ml), Viability (%), Dilution factor and Status.

Notes

To ensure robust and reliable results, the undiluted cell suspension concentration should be in the range of 5×10^5 - 1×10^8 cells/ml. If the cell concentration is above 1×10^8 cells/ml, dilute the original sample in your preferred growth medium. Then count the diluted cell sample as described above. For correct calculation of cell concentrations and viability, remember to update the volume input fields with the new volumes used.
Reagent **Y200** will precipitate below 15°C. Check buffer for precipitation before use. Dissolve any precipitation by warming to room temperature and inverting the container several times.

Viability

The viability percentage is calculated as follows:

$$\%\ \text{Viability} = \frac{C_t - C_{nv}}{C_t} * 100\%$$

% Viability: The percentage of viable cell in the cell sample
C_t: The total concentration of AO positive cells in the first Via2-Cassette™
C_{nv}: The non-viable concentration of DAPI positive cells in the second Via2-Cassette™

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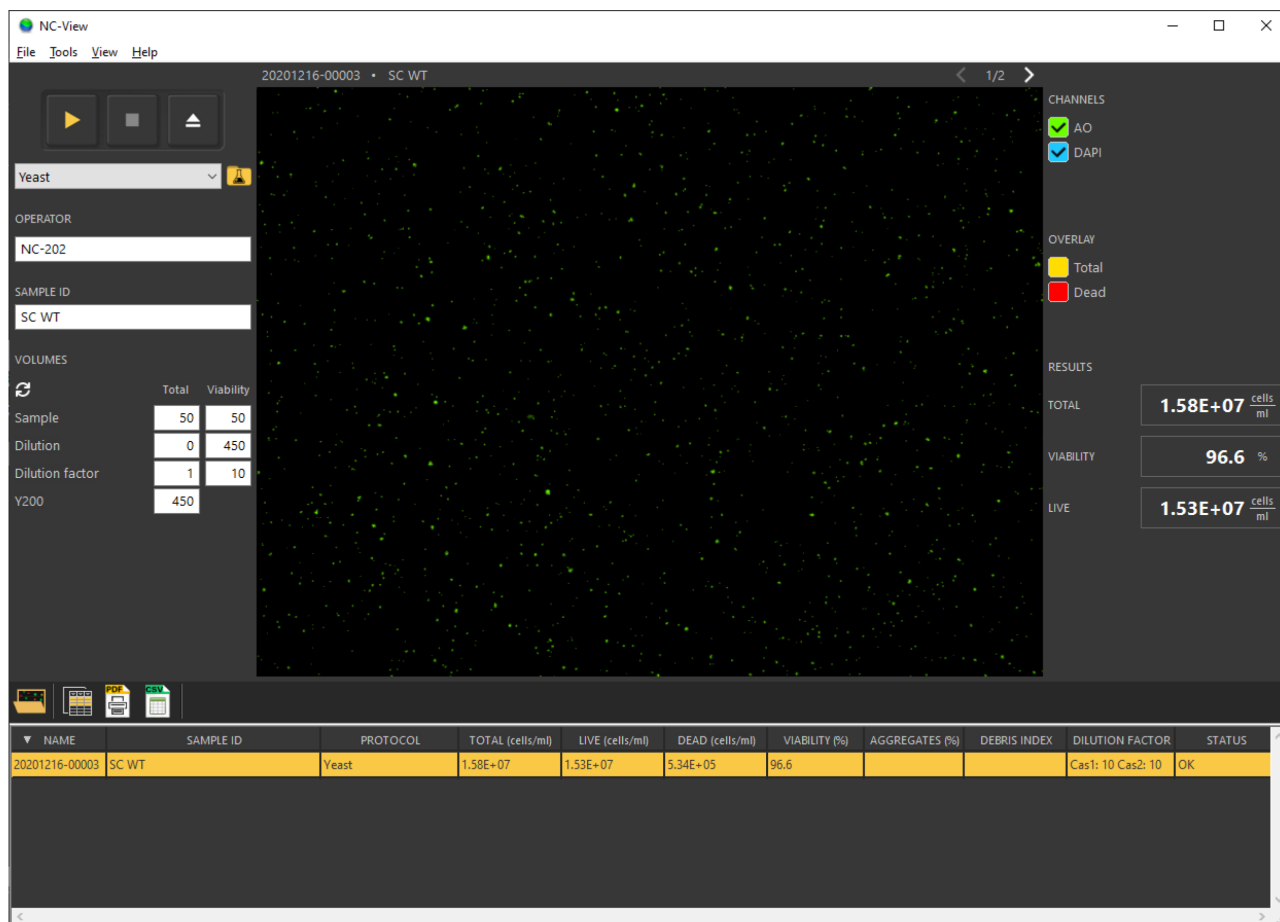

Picture of NC-View™ software after running a Yeast protocol on a Saccharomyces cerevisiae cell sample using the NucleoCounter® NC-202™. Acridine orange (AO) and DAPI channels are shown in green and blue, respectively. Enabling the image overlay displays total (yellow) and dead cells (red) identified by the software. Counting results are presented in the right panel and in the file list below.

Troubleshooting

<u>Inaccurate cell count: My cell count is either too high or low</u>

When analyzing a new cell line, it is important to verify that the cells are correctly identified and recorded. Cells identified by the NC-View™ software can be shown by clicking cell overlay, right panel (see figure). All cells should be highlighted, while cellular debris should be excluded.

<u>Imprecise cell count: I see large variation between technical replicates</u>

The cell counting precision, often quantified as the coefficient of variation from replicate counts, is affected by many variables, including:

1. Cell concentration: A low cell sample concentration will negatively affect the counting precision. See our Technical note: Effects of sample concentration on cell counting variation NucleoCounter® NC-202™ *(document no. 994-2030)*
2. Liquid handling: The cell suspension should be thoroughly mixed before the sample is aspired into the Via2-Cassette™

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3. Cell sample size: The Via2-Cassette™ can aspirate from 200 µl sample in a 1.5 ml tube, however increasing the sample volume improves the precision
4. Consistent protocol execution: Human variation and possibly error in sample handling causes variation between samples and replicas
5. Sample preparation: Ensure that cell sampling and sample dilutions are made using wide orifice tips to avoid 'bottleneck effects'

Viability and live count are negative:

If the concentration of dead cells is more than 10% higher than the total cell concentration a warning will be given in the status column. This can be due to inaccuracies when diluting the cell sample or because the user forgot to update the volume fields correctly before starting the protocol.

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-202™ user 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-202™ system is FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE. The results presented by the NucleoCounter® NC-202™ system depend on correct use of the reagents, Cassettes and the NucleoCounter® NC-202™ instrument and might depend on the type of cells being analyzed. Refer to the NucleoCounter® NC-202™ 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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