Application note No. 300. Rev. 1.0

Total cell count of yeast (Saccharomyces cerevisiae) using the NucleoCounter® YC-100™ system

Product description

The NucleoCounter® YC-100™ system is comprised of the NucleoCounter® YC-100™ instrument, NucleoCassette™ and Reagent Y100. An optional but recommended part of the system is the NucleoView™ software.

The NucleoCounter® YC-100™ is developed as a stand-alone instrument, but it is recommended that the NucleoCounter® YC-100™ is connected to a computer using the NucleoView™ software, which offers a variety of features such as storage of the results and export and printing of the data.

Application

This protocol for the NucleoCounter® YC-100™ system enables the user to obtain absolute volumetric cell count (total cell count) of yeast and thereby to estimate the total number of cells. The protocol is suitable for use on yeast samples, which has low degree of flocculation. It is recommended that NucleoView™ software is used for this application.

Introduction

Biomass and biological product produced during a fermentation process are dependent on both cell number and physiological conditions. Whereas the physiological conditions can be controlled during the process, the cell number is mainly controlled by adjusting the initial cell number at the start of the process. Monitoring the cell number allows the scientist to activate inducible systems or to harvest at optimal times for the best product yield. Processes where transformation of yeast is used, can also benefit from this protocol. Traditionally a measurement of a solutions optical density at 600nm (OD_{600}) provides a rough estimate of the number of yeast cells in suspension, but in general a dry cellular weight (DCW) measurement or volumetric cell count provide the best fundament for consistency and optimization of the culturing process. NucleoCounter® YC-100™ system provides a fast and accurate DNA based volumetric cell count within seconds, independentl of debris or substrate aggregates in the media. The NucleoCounter® YC-100™ is maintenance and calibration free, offering extreme ease of use and reliable operation, and is especially well suited for routine analysis of yeast samples.

It is recommended that users validate the NucleoCounter® YC-100™ method individually for different yeast strains against their own preferred method of reference for estimating the total concentration of cells or cellular mass, e.g. dry cellular weight, counting chamber with manual estimation of cell concentration, flow cytometry, impedance cell counter, or optical density (OD).

Principle

In order to determine the total concentration of yeast cells, the cells are treated with Reagent Y100. Reagent Y100 permeates the yeast cell wall and membranes, thereby allowing the cellular DNA to be stained with propidium iodide, which is safely immobilized on the inside of the NucleoCassette™. Reagent Y100 has the

ability to dissociate minor cell clusters of yeast cells, but not to dissolve or dissociate cells that are in the process of reproductive division.

Approximate correlation between OD, DCW and yeast cell count:

1 OD_{600} (1 unit optical density measured at 600nm, within linear range)
= approx. 0.3 to 0.6 g dry cellular mass/Liter (dry cellular weight, DCW)
= approx. 1×10^6 to 5×10^6 cells/mL

After Reagent Y100 treatment (generally less than 30 seconds), approximately 50 µl of the cell suspension is drawn into the NucleoCassette™ that contains sufficient amounts of propidium iodide for the staining of the cellular DNA. The NucleoCassette™ is placed in the NucleoCounter® YC-100™ where the cells are automatically enumerated within 30 seconds yielding an absolute volumetric cell count. Using the NucleoCounter® YC-100™ system it is possible to determine the total number of yeast cells.

Correlation between OD or CDW and volumetric cell counts:
A correlation curve may be constructed specifically for each type (and strain) of yeast being measured. Thereby it is possible to calculate the approximate corresponding optical density (OD) or approximate dry cellular weight (DCW) from the cell number measurement using the NucleoCounter® YC-100™.

Procedure

Procedure for counting yeast cells in suspension

1. Dispense 450µL Reagent Y100 solution in a 1.5mL polypropylene tube.

2. Mix the sample suspension thoroughly by inverting/shaking the sample tube to ensure homogeneity. Transfer 50µL of sample cell suspension to the reagent solution. Mix the solution well by vortexing (or by pipetting vigorously for a few seconds). The mixture total volume is then 500µL, giving a 10-fold dilution. Incubation times of up to 10 minuttes may be necessary for different types of yeast.

   Note:
   From this point the treated cells suspension may be stored at room temperature or in refrigerator for some time before analysis. Remember always to mix samples well (vortex) before aspirating any volume, or before loading the NucleoCassette™. The storage stability of the diluted samples must be investigated for each type of yeast.

3. If necessary repeat steps 1-2 in order to achieve an additional 10-fold dilution each time. A 100-fold to 1,000-fold dilutiuon, is generally suitable for most terminal industrial yeast fermentations. Alternative dilution with Reagent Y100 may also be used. Incubation times of up to 10 minuttes may be necessary for different types of yeast.

   Note:
   It is recommended to use minimum equal volumes of Reagent Y100 and cell suspension to assure effective cell permeabilization.

Recommended dilution:

OD_{600} (optical density at 600nm)	Approx. recommended dilution
< 0.5	5
0.5 - 1	10
1–5	50
5-10	100
10-15	150
15-20	200
> 20	> 200

Table 1: Recommended approximate dilutions for using the NucleoCounter® YC-100™ on yeast cells. The recommended dilutions is only approximate values, since the optical density may vary for different types and strains of yeast cells. The above mentioned recommended dilution is based on *Saccharomyces cerevisiae* (CEN.PK 113-7D) measurements in the linear range of OD correlation (see Figure 1). The total concentration of cells in the diluted cell suspension should be adjusted to be within the limits of 1×10^5 cells/ml to 2×10^6 cells/ml to ensure a statistically reliable count. OD measurements are instrument specific, and may vary for different spectrophotometer manufacturers.

4. Count cells on NucleoCounter® YC-100™ using the the NucleoCassette™. Remember that the multiplication factor in NucleoView™ should be set correctly according to the total dilution used in the above steps (e.g. 100).

   It is recommended that the cell concentration is measured at least three times (3 different analyses on each sample) for each type and strain of yeast when constructing correlation curves. OD measurements are instrument specific, and may vary for different spectrophotometer manufacturers.

   Note:
   To assure a statistically reliable yeast cell count with the NucleoCounter® YC-100™, it is recommended that the total concentration of cells in the diluted cell suspension should be within 1×10^5 cells/ml to 2×10^6 cells/ml.

5. If the concentration of cells in the undiluted sample is below 1×10^5 cells/ml then the concentration of cells may be increased by centrifugation (>10,000×g, 15min) followed by resuspension of the cell pellet in PBS or growth media before counting.

   The recommended minimum volume of treated cell suspension is 0.5mL per tube before analysis in order to ensure representative amounts of sample. pH

of the treated cell suspension should be between 4 and 9 in order for the propidium iodide to stain the DNA correctly. pH of the Reagent Y100 is approximately 7, but it is not buffered.

Extract cell counts from the NucleoView™ software, e.g. by using the export data utility.

Construction of a correlation curve (optional):
Exported data may be imported into a spreadsheet program. Results may be plotted as

Optical Density (OD_{600}) against Cell concentration (cells/mL)
(see Figure 1)

or Dry Cellular Weight (g/L) against Cell concentration (cells/mL).

For different yeasts a correction multiplication factor (F) between 1 to 1.5 may be used for correlating the NucleoCounter® YC-100™ cell counts to the whole cell counts from different manual counting methods. A portion of the growing yeast cells may form clusters that contain two or more cells, hence leading to a difference between the manual cell counts, and the single cell counts from the NucleoCounter® YC-100™.

Example
Correlation Curve

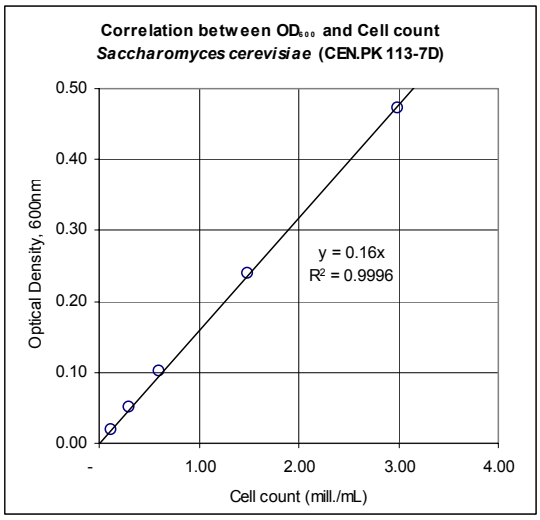

Figure 1: Correlation of OD_{600} and cell counts in the linear range using NucleoCounter® YC-100™. (*Saccharomyces Cerevisiae*, CEN.PK 113-7D) . Generally OD should be measured within the linear range of correlation.

Image Example

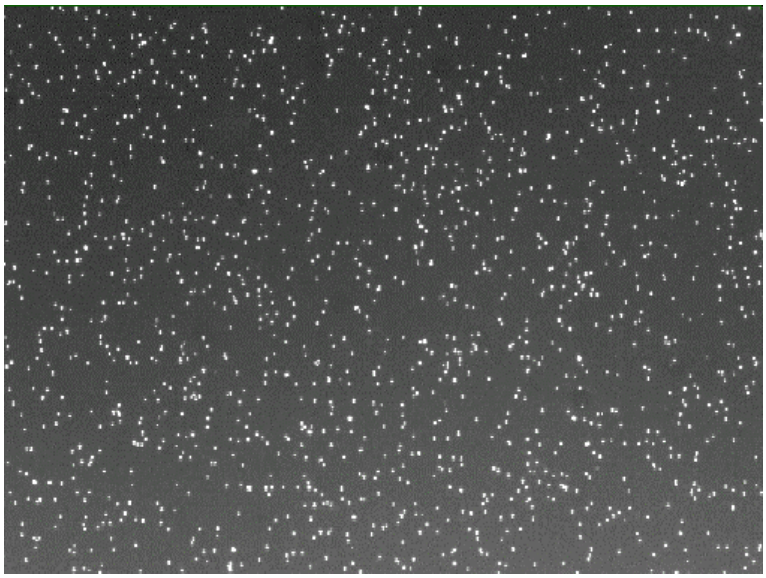

Figure 2: NucleoCounter® YC-100™ raw image of a yeast cells suspension (Saccharomyces Cerevisiae) after Reagent Y100 treatment using 100-fold dilution. Cell count is approx. 8×10^7/mL. The objects have simlar fluorescence intensities and are uniform in size, indicating minimal cell disruption. The yeast cells are uniformly dispersed throughout the entire image. The yeast cells may be manually monitored in the NucleoCassette™ measurement chamber using fluorescence microscopy with apropriate filters for propidium iodide.

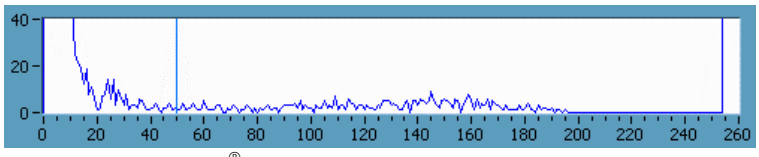

Figure 3: NucleoCounter® YC-100™ object fluorescence intensity data plotted in a frequency histogram ("object intensity histogram"). Correctly identified fluorescent objects that fall within the "gate", between the vertical dicriminator line (discriminator=50) and the maximum signal value of 255, are counted as cells. Most of the identified cells have one or two pixels in saturation (data not shown). The process of counting cells in the image by the built-in computer, is fully automated in the NucleoCounter® YC-100™ instrument.

Note:
To assure a statistically reliable yeast cell count with the NucleoCounter® YC-100™, it is recommended that the total concentration of cells in the diluted cell suspension should be within 1×10^5 cells/ml to 2×10^6 cells/ml.

Appendix

Determination of generation (or doubling) time, g, and mean growth constant, k, for exponential growth of yeast

The increase in cell number that occurs in an exponentially growing cell culture is a geometric progression of the 2nd order. Because of this geometric progression, there is a direct relationship between the number of cells present in a culture at the beginning (t=0) of the logarithmic phase and the number present after a period of exponential growth;

$$N = N_0 \times 2^n$$

where N is the final number of cells per volume [mL^{-1}],
N_o is the initial number of cells per volume [mL^{-1}],
n is the number of generations,
where one generation is defined as the time when cell count is doubled.

After transformation, the number of generations is

$$n = \frac{\ln N - \ln N_o}{\ln 2}$$

The generation time (or doubling time), g, is defined as

$$g = \frac{t}{n}$$

and therefore

$$g = \frac{t \times \ln 2}{\ln N - \ln N_o}$$

where t is the time of exponential growth in relevant dimension [min] or [h] between the two measurements.

The mean growth constant, k, that is the number of divisions per unit time, can be found as

$$k = \frac{1}{g}$$

References

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Methods in Yeast Genetics: A Laboratory Manual.
Cold Spring Harbor Laboratory Press, New York

Prescott LM, Harley JP, Klein DA (1999)
Microbiology (fourth edition)
WBC/ McGraw-Hill, New York

Campbell I, Duffus JH (1996)
Yeast - A Practical Approach
Oxford University Press, IRL Press, Washington DC

Handling and storage

For handling and storage of ChemoMetec instruments and reagents and NucleoCassettes™ 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 NucleoCassettes™ refer to the corresponding product documentation and the NucleoCounter® YC-100™ 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® YC-100™ system is FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE. The results presented by the NucleoCounter® YC-100™ system depend on correct use of the reagents, NucleoCassettes™ and the NucleoCounter® YC-100™ and might depend on the strain of cells being analysed. Refer to the NucleoCounter® YC-100™ 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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Technology that counts