Application note 004 Ver. 1.1

Single-cell-suspension

Cell counts and viability – Reagent C100

Product description

The NucleoCounter™ system is comprised of the NucleoCounter™, NucleoCassettes™, Lysis buffer and Stabilizing buffer. An optional part of the system is the NucleoView™ software.

Reagent C100

Lysis buffer C100 is a 1:1 mixture of the Lysis buffer A100 and the Stabilizing buffer B. Reagent A100 is an aqueous solution of inorganic acid and surfactant. Reagent B is an alkaline aqueous solution of surfactant and inorganic base. The pH value of the final Lysis buffer C100 is 4.1.

The Lysis buffer C100 has proven sufficient for lysing single-cell-suspension cells, since they do not require de-clumping.

Application

The NucleoCounter system enables the user to obtain absolute cell counts (total and non-viable) and thereby determine the viability of cell suspensions from a wide range of cultured mammalian cells.

The NucleoCounter is developed as a stand-alone instrument. Optionally the NucleoCounter can be connected to a computer using the NucleoView software, which offers a variety of features such as documentation of the results and calculation of viability.

Principle

In order to determine the total concentration of cells in a single-cell-suspension of mammalian cells, a sample of the suspension is treated with the Lysis buffer C100. The objective of the Lysis buffer is to permeate the plasma membranes, thereby allowing the nuclei to be stained with propidium iodide, which is coated on the inside of the NucleoCassette. The Lysis buffer C100 does not have the ability to dis-aggregate cell clusters. The pH value of the resultant cell lyzate is optimal for propidium iodode to stain the nuclei efficiently. Approximately 50 µl of the cell lyzate is then drawn into the NucleoCassette. The NucleoCassette is placed in the NucleoCounter where the cells are counted.

To obtain the concentration of non-viable cells the cell suspension is loaded directly into the NucleoCassette without any pre-treatment. Thereby, only the non-viable cells (with impaired plasma membranes) are stained with propidium iodide and counted.

Using the NucleoCounter system it is possible to determine the total concentration of cells as well as the concentration of non-viable cells. Subsequently, the viability may be calculated.

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Procedure

First, the procedure for determining the total concentration of cells is described. Thereafter, the procedure for determining the concentration of non-viable cells is described. Finally, the method for calculating the viability of cells on the basis of the two mentioned measurements is shown.

Total cell count

Mix a stock solution of Reagent C100:

Mix 1 part of Lysis buffer A100 with 1 part of Stabilizing buffer B. (e.g. 5 ml of A100 + 5 ml of B).

Count the total concentration of cells in the cell suspension:

1. Mix 1 part of the mammalian single-cell-suspension with 2 parts of the Lysis buffer C100. Note: You can use any volume of Lysis buffer C100, dependent on the concentration of cells in the cell suspension. See notes below.
2. Mix thoroughly (use a vortex mixer).
3. Draw a sample of the cell lyzate by inserting the tip of the NucleoCassette into the lyzate and pressing the piston.
4. Insert the NucleoCassette into the NucleoCounter, close the lid and press RUN. After approximately 30 seconds the total concentration of nuclei in the cell lyzate is displayed on the NucleoCounter and on the computer if one is connected to the NucleoCounter. Note, that it is the number of cells pr. ml in the cell lyzate drawn into the NucleoCassette, which is displayed. Therefore, in order to calculate the number of cells pr. ml in the original suspension, the displayed number must be multiplied with the multiplication factor. The multiplication factor is 3 assuming the suspension of cells has not been diluted prior to treatment with Lysis buffer and the procedure above has been followed.

Non-viable cell count

1. Draw a sample of the single-cell-suspension into the Nucleocassette. The cells must not be treated with Lysis buffer prior to counting the number of non-viable cells.
2. Insert the NucleoCassette into the NucleoCounter, close the lid and press RUN. After approximately 30 seconds the total concentration of nuclei in the cell lyzate is displayed on the NucleoCounter and on the computer if one is connected to the NucleoCounter.

Note

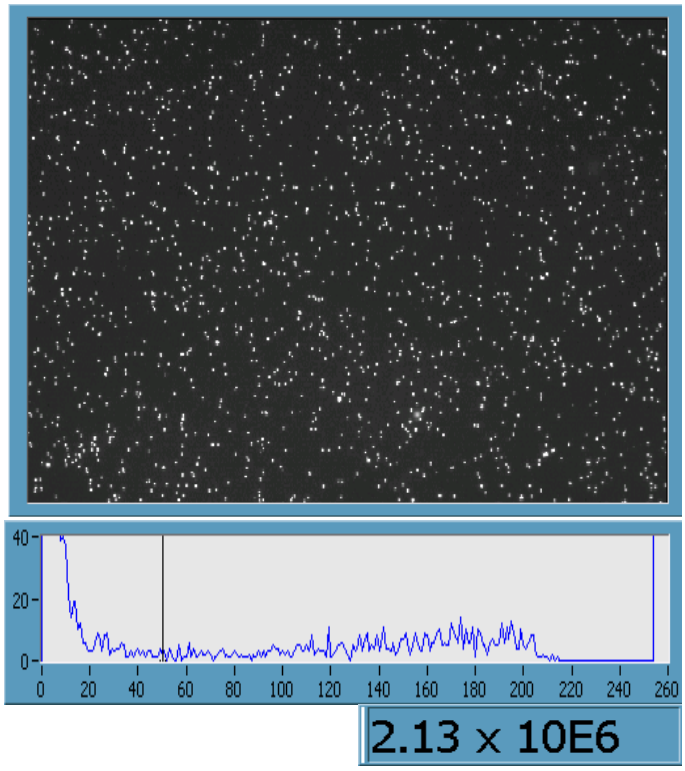
To assure reliable cell counts with the NucleoCounter, it is recommended that the total concentration of cells in the cell lyzate should be within the limits of $5 \cdot 10^3$ cells/ml to $2 \cdot 10^6$ cells/ml. To obtain objective viability results it is recommended that Total count concentrations should be > 1x10E5.

If the concentration of cells in the cell lyzate is below $5 \cdot 10^3$ cells/ml then the concentration of cells per volume may be increased by centrifugation followed by re-suspension of the pellet using PBS or growth media.

If the concentration of cells in the cell lyzate is above $2 \cdot 10^6$ cells/ml, the suspension of cells can be diluted with PBS or growth media to achieve the desired concentration. Hereafter, the suspension is treated with Lysis buffer.

Note

When counting the number of non-viable cells, the cells are not pre-treated with Lysis buffer and they are thereby not diluted. Thus, the number displayed on the NucleoCounter and/or the computer is the actual number of non-viable cells pr. ml in the suspension. If the suspension of cells has been up-concentrated or diluted the appropriate multiplication factor must be used to calculate the correct concentration of non-viable cells in the original cell suspension.



Example of a total cell count using Lysis buffer C100. YAC-1 cells were diluted three times with Lysis buffer C100. (E.g. 400 µl Lysis buffer C100 + 200 µl YAC-1 cell suspension with a concentration of 2.13 cells pr. ml.).

Viability

It is possible to calculate the viability of the cells after having obtained the total concentration of cells and the concentration of non-viable cells. Assuming that the cells have been counted as described above the calculation of viability is as follows.

$$\%viability = \frac{C_t \cdot M_t - C_{nv} \cdot M_{nv}}{C_t \cdot M_t} \cdot 100\%$$

%viability	The percentage of viable cells in the original cell suspension.
C_t	The total concentration of cells in the NucleoCassette (the displayed result of the total cell count).
C_{nv}	The concentration of non-viable cells in the NucleoCassette (the result displayed when counting the non-viable cells).
M_t	The multiplication factor used for the total cell count (most often 3).
M_{nv}	The multiplication factor used for the non-viable cell count (most often 1).

Handling and storage

For handling and storage of reagents and NucleoCassettes refer to the individual packing labels.

Warnings and precautions

For safe handling and disposal of the reagents and NucleoCassettes refer to the packing labels and the NucleoCounter user's guide.

Limitations

The NucleoCounter system is for research use only. The results presented by the NucleoCounter system depend on correct use of the reagents, NucleoCassettes and the NucleoCounter. Refer to the NucleoCounter user's guide for instructions and limitations.

Intended use

The Nucleocounter™ system is for research use only – not for diagnostic use.

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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