Application note 002

Cell counts and viability – Reagent A100

Product description

The NucleoCounter™ system is comprised of the NucleoCounter™, NucleoCassettes™, Lysis buffer (Reagent A100 or Reagent A) and Stabilizing buffer (Reagent B). An optional part of the system is the NucleoView™ software.

Reagent A100

The Lysis buffer, Reagent A100, has been introduced as an alternative to Reagent A, as some cell lines, - under special applications, has proved to be sensitive to Reagent A.

Reagent A100 is an aqueous solution of inorganic acid and surfactant. The pH value is 1.25. Despite the low pH value Reagent A100 is considered a milder Lysis buffer than Reagent A. Furthermore the declumping abilities has been improved significantly.

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 suspension of mammalian cells, a sample of the suspension is first treated with Lysis buffer and then Stabilizing buffer. 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 also contributes to the disaggregation of cell clusters. The following treatment with Stabilizing buffer raises the pH value of the mixture in order to allow propidium iodide to stain the nuclei more efficiently. Approximately 50 µl of the stabilized 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 with Lysis buffer or Stabilizing buffer. 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.

Procedure

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

Technology that counts

Total cell count

1. Mix 1 part of mammalian cells in suspension with 1 part of Lysis buffer (e.g. 200 µl cell suspension and 200 µl Lysis buffer).
2. Mix thoroughly (use a vortex mixer).
3. Add 1 part of Stabilizing buffer (e.g. 200 µl) to the mixture, which is now called the stabilized lyzate.
4. Mix thoroughly (use a vortex mixer).
5. Draw a sample of the stabilized lyzate by inserting the tip of the NucleoCassette into the lyzate and pressing the piston.
6. Insert the NucleoCassette into the NucleoCounter, close the lid and press RUN. After approximately 30 seconds the total concentration of nuclei in the stabilized 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 stabilized lyzate drawn into the NucleoCassette, which is displayed. Therefore, in order to calculate the number of mammalian cells pr. ml in the original suspension, the displayed number must be multiplied by the multiplication factor. The multiplication factor is 3 assuming the suspension of cells has not been diluted prior to treatment with Lysis buffer and Stabilizing buffer and the procedure above has been followed.

Non-viable cell count

1. Draw a sample of the suspension of mammalian cells into the NucleoCassette. The cells must not be treated with Lysis buffer and Stabilizing buffer prior to counting the number of non-viable cells.

Insert the NucleoCassette into the NucleoCounter, close the lid and press RUN. After approximately 30 seconds the concentration of non-viable cells in the suspension of cells is displayed on the NucleoCounter and 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 stabilized 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 stabilized lyzate is below $5 \cdot 10^3$ cells/ml then the concentration of cells per volume may be increased by centrifugation followed by resuspension of the pellet using PBS or growth media.

If the concentration of cells in the stabilized 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 and Stabilizing buffer.

Note

When counting the number of non-viable cells, the cells are not pretreated with Lysis buffer and Stabilizing 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.

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.

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

% 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 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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