

Application note 003

Dilution of the samples

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.
Application	<p>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.</p> <p>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.</p>
Principle	<p>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.</p> <p>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.</p> <p>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.</p>
Dilution of the samples	<p>The measuring range of the NucleoCounter system is $5 \cdot 10^3 - 2 \cdot 10^6$ cells/mL. Therefore, if the concentration of cells in the cell suspension is high (concentration higher than $6 \cdot 10^6$ cells/mL) it has to be diluted before counting the cells using the NucleoCounter. This application note describes two recommended methods for dilution of a cell suspension.</p> <p>The cell suspension can be diluted before the sample is drawn; pre-dilution of the cell suspension. Alternatively the lyzate (cell sample treated with Lysis buffer and Stabilizing buffer) can be diluted e.g. if the sample material is limited and no more sample can be drawn; dilution of the lyzate.</p>

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Pre-dilution of the cell suspension

When the concentration of cells in a cell suspension is high it can be diluted prior to the treatment with Lysis buffer and Stabilizing buffer. As dilution media use cell media, PBS or another balanced salt solution. Use the table below when diluting a cell suspension.

Expected cell concentration (cells/mL)	Cell suspension	Cell media or PBS ¹	External multiplication factor	Diluted cell sample, Lysis buffer, Stabilizing buffer	Multiplication factor
1.5·10 ⁴	1	0	0	1, 1, 1	3
5.0·10 ⁶	1	0	0	1, 1, 1	3
1.0·10 ⁷	1	1	2	1, 1, 1	6
1.5·10 ⁷	1	2	3	1, 1, 1	9
2.0·10 ⁷	1	3	4	1, 1, 1	12
2.5·10 ⁷	1	4	5	1, 1, 1	15
3.0·10 ⁷	1	5	6	1, 1, 1	18
3.5·10 ⁷	1	6	7	1, 1, 1	21
4.0·10 ⁷	1	7	8	1, 1, 1	24
4.5·10 ⁷	1	8	9	1, 1, 1	27
5.0·10 ⁷	1	9	10	1, 1, 1	30
5.5·10 ⁷	1	10	11	1, 1, 1	33
6.0·10 ⁷	1	11	12	1, 1, 1	36
6.5·10 ⁷	1	12	13	1, 1, 1	39
7.0·10 ⁷	1	13	14	1, 1, 1	42
7.5·10 ⁷	1	14	15	1, 1, 1	45
8.0·10 ⁷	1	15	16	1, 1, 1	48
8.5·10 ⁷	1	16	17	1, 1, 1	51
9.0·10 ⁷	1	17	18	1, 1, 1	54
9.5·10 ⁷	1	18	19	1, 1, 1	57
1.0·10 ⁸	1	19	20	1, 1, 1	60

¹ Use cell media, PBS or another balanced salt solution.

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The table above shows the recommended way to pre-dilute concentrated cell suspensions. The aim is that the concentration of cells inside the NucleoCassette is approximately $1.67 \cdot 10^6$ cells/mL.

The first column **Expected cell concentration (cells/mL)** denotes the expected concentration of cells in the cell suspension, which is about to be analyzed. Through this column the exact way to pre-dilute the cell suspension is found. An example: if the expected cell concentration is between $1.0 \cdot 10^7$ and $1.5 \cdot 10^7$ cells/mL the way to dilute the cell suspension is found in the row where the expected cell concentration is $1.5 \cdot 10^7$ cells/mL.

The next two columns, **Cell suspension** and **Cell media or PBS¹**, denotes how to mix a sample from the cell suspension with cell media or PBS to obtain the desired cell concentration. The starting point is an expected cell concentration of $1.5 \cdot 10^7$ cells/mL. According to the table 1 part of cell suspension is mixed with 2 parts of cell media or PBS. This could be 200- μ L cell suspension and 400 μ L cell media or PBS.

The **External multiplication factor** (external because the dilution is performed before treatment with Lysis buffer and Stabilizing buffer) results from mixing the cell suspension with cell media or PBS. In this example the external multiplication factor is 3, as 1 part cell suspension is mixed with 2 parts cell media or PBS resulting in a diluted cell sample.

Before analyzing the diluted cell sample on the NucleoCounter, it is treated with Lysis buffer and Stabilizing buffer. The column **Diluted cell sample, Lysis buffer, Stabilizing buffer** shows how to treat the diluted cell sample with Lysis buffer and Stabilizing buffer. Add 1 part diluted cell sample (e.g. 200 μ L) to 1 part Lysis buffer (e.g. 200 μ L) and mix thoroughly. Add 1 part Stabilizing buffer (e.g. 200 μ L) and mix again. This procedure is the same throughout the column.

The last column is **Multiplication factor**, which denotes the overall multiplication factor. When a result is presented on the NucleoCounter after analysis, multiply the concentration of cells in the NucleoCassette with the multiplication factor to obtain the concentration of cells in the cell suspension. The calculation is performed automatically in the NucleoView software; just remember to adjust the multiplication factor.

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Dilution of the lyzate

Dilution of the lyzate is an alternative to dilution of the cell suspension. It can come in handy if the expected cell concentration is not known. Then it is possible to dilute the lyzate after the first analysis is performed. The table below shows how to prepare and dilute the lyzate.

Expected cell concentration (cells/mL)	Cell sample, Lysis buffer, Stabilizing buffer	Lyzate, Mix of Lysis buffer and Stabilizing buffer ²	Multiplication factor
$1.5 \cdot 10^4$	1, 1, 1	0	3
$5.0 \cdot 10^6$	1, 1, 1	0	3
$1.0 \cdot 10^7$	1, 1, 1	1, 1	6
$1.5 \cdot 10^7$	1, 1, 1	1, 2	9
$2.0 \cdot 10^7$	1, 1, 1	1, 3	12
$2.5 \cdot 10^7$	1, 1, 1	1, 4	15
$3.0 \cdot 10^7$	1, 1, 1	1, 5	18
$3.5 \cdot 10^7$	1, 1, 1	1, 6	21
$4.0 \cdot 10^7$	1, 1, 1	1, 7	24
$4.5 \cdot 10^7$	1, 1, 1	1, 8	27
$5.0 \cdot 10^7$	1, 1, 1	1, 9	30
$5.5 \cdot 10^7$	1, 1, 1	1, 10	33
$6.0 \cdot 10^7$	1, 1, 1	1, 11	36
$6.5 \cdot 10^7$	1, 1, 1	1, 12	39
$7.0 \cdot 10^7$	1, 1, 1	1, 13	42
$7.5 \cdot 10^7$	1, 1, 1	1, 14	45
$8.0 \cdot 10^7$	1, 1, 1	1, 15	48
$8.5 \cdot 10^7$	1, 1, 1	1, 16	51
$9.0 \cdot 10^7$	1, 1, 1	1, 17	54
$9.5 \cdot 10^7$	1, 1, 1	1, 18	57
$1.0 \cdot 10^8$	1, 1, 1	1, 19	60

² Use a mixture of equal volumes of Lysis buffer and Stabilizing buffer. Ensure that the Lysis buffer and Stabilizing buffer are mixed thoroughly before adding it to the lyzate.

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The table above shows the way to dilute the lysate if the performed analysis resulted in a cell count higher than $2 \cdot 10^6$ cells/mL in the NucleoCassette and no other sample can be drawn from the cell suspension or if this way of dilution is preferred.

Again the **Expected cell concentration (cells/mL)** is shown in the first column. Use it the same way as above to find the row stating the exact dilution procedure.

The second column **Cell sample, Lysis buffer, Stabilizing buffer** is the same throughout the table. It shows how to treat the cell sample with Lysis buffer and Stabilizing buffer. Draw 1 part cell sample (e.g. 200 μ L) and mix it thoroughly with 1 part Lysis buffer (e.g. 200 μ L). Add 1 part Stabilizing buffer (e.g. 200 μ L) and mix again. The lysate is now ready for dilution.

The way to mix the lysate with a mix of Lysis buffer and Stabilizing buffer² is shown in the column **Lysate, Mix of Lysis buffer and Stabilizing buffer**. An example: if the expected cell concentration is between $1.0 \cdot 10^7$ and $1.5 \cdot 10^7$ cells/mL, the cell sample is first treated with Lysis buffer and Stabilizing buffer as described above. Then 1 part lysate is mixed with 2 parts of the mix of Lysis buffer and Stabilizing buffer. To do this draw a sample of the lysate (e.g. 200 μ L) and add 2 parts of the mix of Lysis buffer and Stabilizing buffer to the lysate (400 μ L). Mix thoroughly and analyze the diluted lysate on the NucleoCounter.

The overall **Multiplication factor** is stated in the last column. When the result is presented on the NucleoCounter after analysis, multiply the concentration of cells in the NucleoCassette with the multiplication factor to obtain the concentration of cells in the cell suspension. The calculation is performed automatically in the NucleoView software; just remember to adjust the multiplication factor.

Note	It might not be necessary to dilute a sample for a non-viable cell count even if the corresponding sample for the total cell count has been diluted.
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.
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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