Application Note No. 2028. Rev. 1.0

NucleoCounter® NC-202™

Lysis Count - Via2-Cassette™

Product description

The NucleoCounter® NC-202™ system performs viability and cell counting on a broad range of mammalian cells.

Application

The Via2-Cassette™ and **Lysis 1** used together with the NucleoCounter® NC-202™ allow for determination of the viability and concentration of aggregating cells. Treatment of cell samples with **Lysis 1** facilitates disaggregation and lysis of cell aggregates resulting in single cell suspensions. Moreover, **Lysis 1** enables accurate enumeration of large cells, such adipocytes, and detachment of anchorage dependent cells, e.g. cells growing on micro carriers.

Introduction

Determination of both cell count and viability requires two Via2-Cassettes™. For measuring the total cell concentration, a sample containing cells in suspension is diluted with **Lysis 1** and loaded into the first Via2-Cassette™, thereby staining the cell nuclei with DAPI. The second Via2-Cassette™ determines the viability by staining the total and non-viable cell population with, respectively, Acridine Orange and DAPI.

Image and data are acquired by placing the Via2-Cassettes™ in the NucleoCounter® NC-202™ and executing the Lysis Count script. Viability and cell concentration data are automatically analyzed and presented.

Based on the total cell concentration from the first Via2-Cassette™ and the viability reading from the second Via2-Cassette™ the concentration of the live and dead cells is calculated.

If only information about the total cell concentration is required, then the assay can be stopped after analysis of the first Via2-Cassette™.

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Procedure

Adherent or semi-adherent cells are released from the cell culture surface (e.g. by trypsin). For optimal counting precision use at least 300 µl cell sample and use 1.5 ml micro centrifuge tube, which inner shape fits with the Via2-Cassette™ tip.

Materials needed

1. Cell sample in suspension
2. **Lysis 1**
3. Via2-Cassette™

Procedure

Step 1: Determine the total cell concentration from a cell sample mixed 1:1 with **Lysis 1**.

- The cell suspension is mixed to obtain a homogenous suspension and a sample is mixed 1:1 with **Lysis 1**. E.g., to 100 µl of cell suspension add 100 µl of **Lysis 1**. Mix by pipetting. Load a cell sample by inserting the tip of the first Via2-Cassette™ into the diluted cell suspension and pressing the piston.
- Insert the loaded Via2-Cassette™ in the NucleoCounter® NC-202™, select the '**C&V Lysis-Count' protocol**, press RUN and then Select '**OK**'.

Step 2: Determine the viability of an untreated cell sample.

- The cell suspension (**without Lysis 1** treatment) is mixed again to obtain a homogenous suspension. Draw a cell sample by inserting the tip of the second Via2-Cassette™ into the cell suspension and pressing the piston.
- When the message box requests it, replace the first Via2-Cassette™ with the second Via2-Cassette™ loaded with the cell suspension without addition of **Lysis 1** and select '**OK**'.

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 (%), Diameter (µm), Aggregates (%), Debris Index, Dilution factor and Status.
If the viability reading is not necessary, then press 'cancel' after analysis of the first Via2-Cassette™, whereby the total cell concentration will be provided.

Notes

To ensure robust and reliable results, the cell suspension concentration should be in the range of $5{\cdot}10^4$ to $1{\cdot}10^7$ cells/ml. If the cell concentration is above $1{\cdot}10^7$ cells/ml dilute with growth media. The diluted cell sample is then counted as described above.
Lysis 1 will precipitate below 15°C. Check buffer for precipitation before use. Re-dissolve any precipitation by warming to room temperature.

Viability

The percent viability is calculated from:

$$\%\ \text{Viability} = \frac{\text{Ct} - \text{Cnv}}{\text{Ct}} * 100\%$$

% Viability: The Percentage of viable cell in the cell sample

C_t: The total concentration of cells (Acridine Orange positive cells in the second Via2-Cassette™)

C_{nv}: The concentration of non-viable cells (DAPI positive cells in the second Via2-Cassette™)

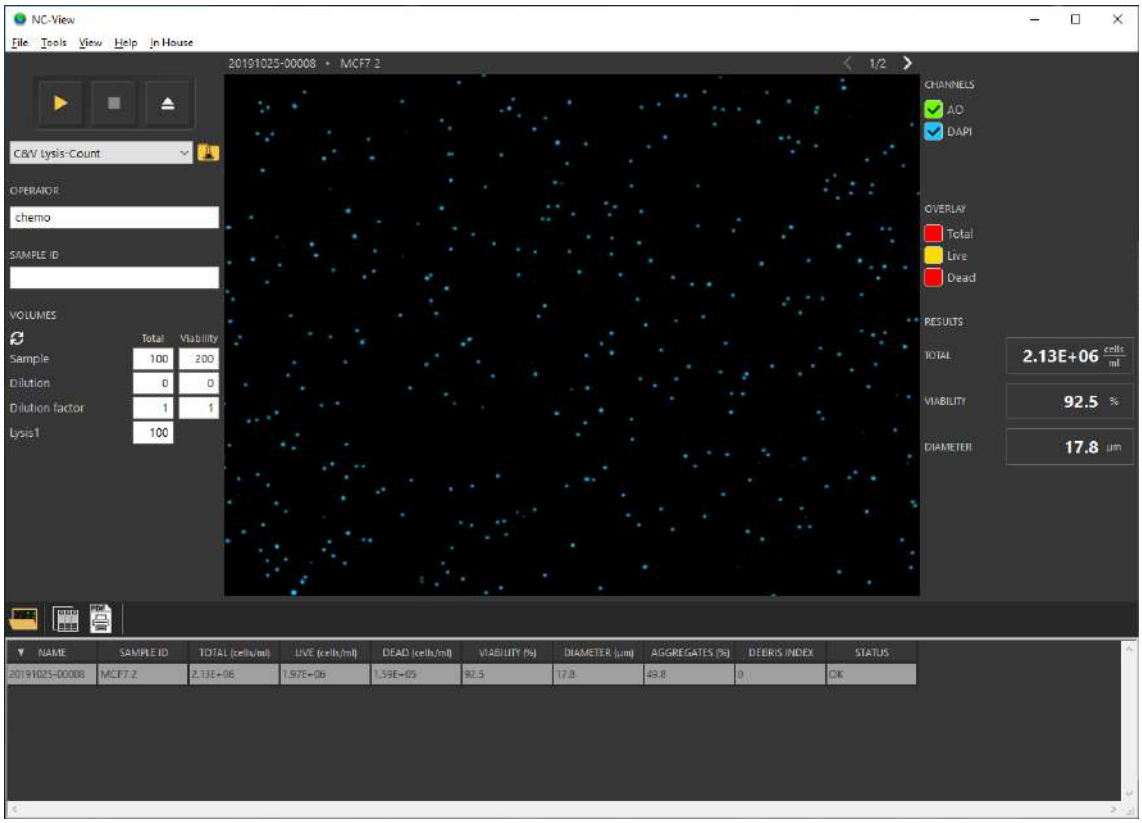

Figure 1: Cell count and viability of disaggregated MCF7 cells using the NucleoCounter® NC-202™. The cells for the first Via2-Cassette™ were disaggregated by adding **Lysis 1** and the cells for the second Via2-Cassette™ were untreated. For the first Via2-Cassette™, the total cell population is stained with DAPI and appears blue in the acquired image. The counting results are presented in the right panel and below in the file list.

Troubleshooting

Inaccurate cell count (my cell count is either too high or low):

When analyzing a new cell line, it is important to verify that the cells are correctly identified. Cells identified by NC-View™ can be shown by clicking cell overlay, right panel (Figure 1). All cells should be highlighted, while cellular debris should be excluded. Note that the 'Total' overlay marks the cells identified in the first Via2-Cassette™, and that the 'Live' and 'Dead' overlays mark the cells identified in the second Via2-Cassette™.

Imprecise cell count (I have a large variation between technical replicates):

The cell counting precision, often quantified as Coefficient of Variation from replicate counts, are affected by many variables. 1) Cell concentration: A low cell sample concentration will negatively affect the precision. See Technical note: *Variation and Statistics*. 2) Liquid handling: The cell suspension should be thoroughly mixed before the sample is collected with the Via2-Cassette™. 3) The cassette can load from 200 µl sample in a 1.5 ml tube, however increasing the sample volume improves the precision. 4) Consistent protocol execution. 5) Sample preparation: Ensure that cell sampling and sample dilutions are made with wide orifice tips, to avoid 'bottleneck effects'.

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 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
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