Application Note No. 2028

NucleoCounter® NC-202™

Aggregated Cells - Via2-Cassette™

Product description

The NucleoCounter® NC-202™ automated cell counter and NC-View™ software perform cell counting and viability on a broad range of eukaryotic cells.

Application

This application note describes how to determine the cell concentration and viability of aggregated mammalian cells, using the **Lysis 1** reagent and Via2-Cassette™. Treating cell samples with **Lysis 1** facilitates disaggregation and lysis of cell aggregates, resulting in a suspension of released single nuclei. Moreover, **Lysis 1** enables accurate enumeration of large cells, such as adipocytes, and detachment of anchorage-dependent cells.

Introduction

Determination of both cell count and viability of aggregated cells requires two Via2-Cassettes™. To measure the total cell concentration, mix the aggregated cell sample with **Lysis 1** and load it into one Via2-Cassette™, thus disassociating and staining the cell nuclei with DAPI. Determine cell viability with a second Via2-Cassette™ which stains the total and non-viable cell population with acridine orange (AO) and DAPI, respectively. Place the Via2-Cassette™ in the NucleoCounter® NC-202™ and press RUN. The NC-View™ software automatically analyses and presents cell concentration and viability for fast and easy data acquisition. If only requiring information about the total cell concentration, analyze the first Via2-Cassette™ only.

Procedure

Release adherent or semi-adherent cells from the cell culture surface by trypsin/EDTA-treatment. For optimal analysis, ≥ 300 µl cell sample is required, preferably in the optimal culture medium for the specific cell type. Transfer a representative sample to a 1.5 ml microcentrifuge tube from which an aliquot can be drawn using the Via2-Cassette™. Treat the sample with **Lysis 1** buffer to lyse the cell membrane, producing a suspension of released single nuclei.

Materials needed

- Cell sample in suspension
- **Lysis 1**
- Via2-Cassette™

Procedure

1. Determine the total cell concentration from a cell sample mixed 1:1 with **Lysis 1**:
   a. Pipette the cell suspension to obtain a homogenous suspension, then mix 1:1 with **Lysis 1** (e.g., add 100 µl of **Lysis 1** to 100 µl of cell suspension). Mix by pipetting
   b. Load a cell sample by inserting the tip of the first Via2-Cassette™ into the mix and press the piston
   c. Insert the Via2-Cassette™ into the NucleoCounter® NC-202™, select the 'Aggregated Cells' protocol and press RUN

2. Determine the viability from an untreated cell sample:
   a. Mix cell suspension (**without Lysis 1**-treatment) obtain a homogenous suspension. Insert the tip of the second Via2-Cassette™ into the suspension and load cell sample by pressing the piston
   b. When prompted by the NC-View™ message box, replace the first Via2-Cassette™ with the second, and select 'OK'

   NOTE: If the viability reading is not necessary, select 'Cancel' after analysis of the first Via2-Cassette™, whereby only the total cell concentration will be provided.

Within one minute, the cell concentration and viability of the sample are displayed. The available results are: Total (cells/ml), Live (cells/ml), Dead (cells/ml), Viability (%), Diameter (µm), Aggregates (%; cell clumps of 5 cells or more), DebrisIndex™, Dilution factor and Status.

Notes

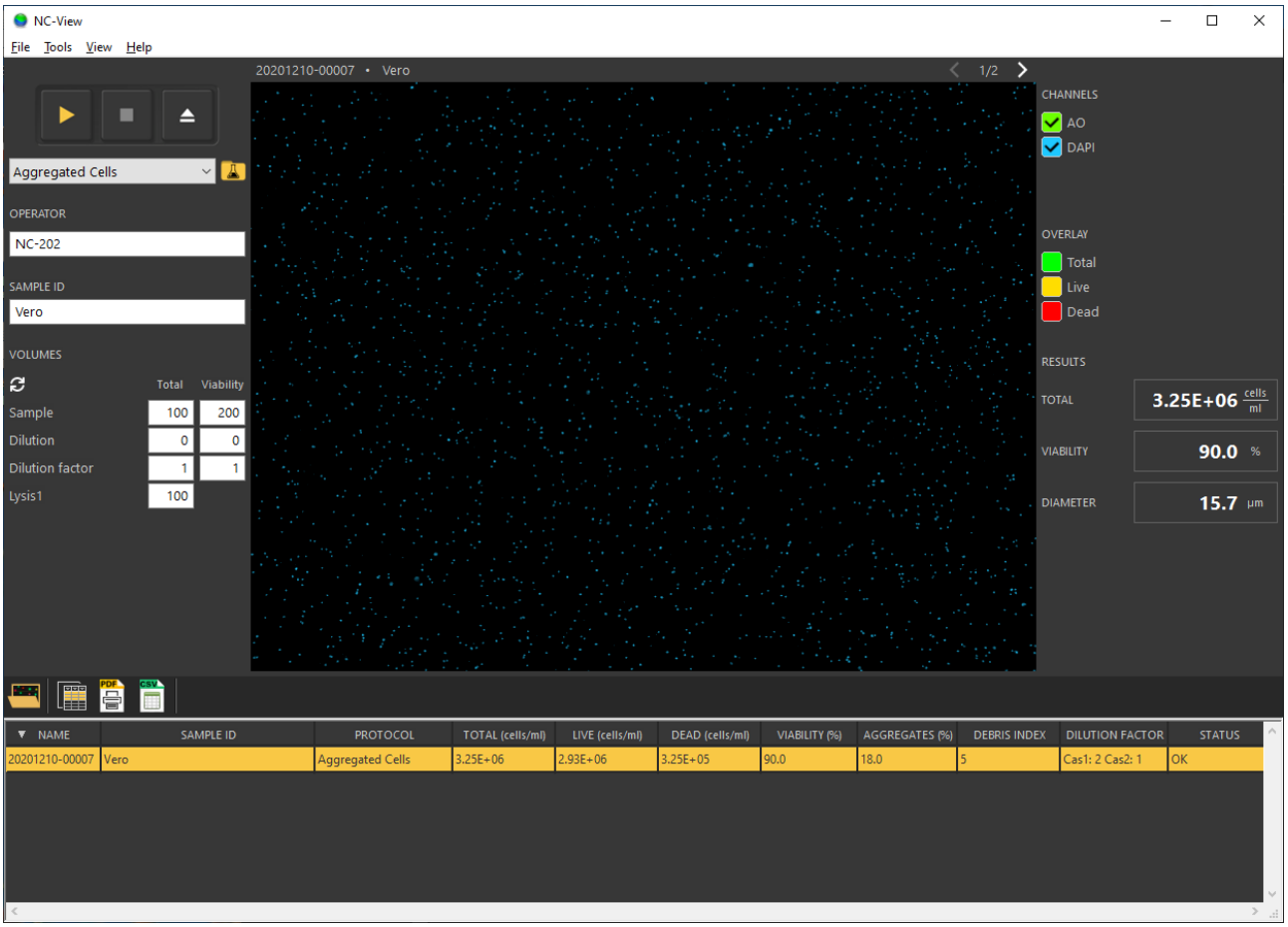
To ensure robust and reliable results, the cell suspension concentration should be in the range of 5×10^4 - 1×10^7 cells/ml. If the cell concentration is above 1×10^7 cells/ml, dilute sample using growth medium, then count as described above. **Lysis 1** will precipitate below 15°C. Check buffer for precipitation before use. Re-dissolve any precipitation by warming to room temperature.
The Diameter (µm) is an estimate based on the AO signal in single cells. The diameter result is therefore subject to some degree of uncertainty and may be affected by cell health, cell density, etc.

Viability

The viability percentage is calculated as follows:

$$\%\ \text{Viability} = \frac{C_t - C_{nv}}{C_t} * 100\%$$

% Viability: The percentage of viable cell in the cell sample
C_t: The concentration of AO positive cells in the second Via2-Cassette™
C_{nv}: The non-viable concentration of DAPI positive cells in the second Via2-Cassette™



Picture of NC-View™ software after running an Aggregated Cells protocol on a Vero cell sample using the NucleoCounter® NC-202™. Acridine orange (AO) and DAPI channels are shown in green and blue, respectively. Enabling the image overlay displays total (green) in view 1, live (yellow), and dead cells (red) in view 2 identified by the software. Counting results are presented in the right panel and in the file list below.

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 identified and recorded correctly. Cells identified by the NC-View™ software can be shown by clicking cell overlay, right panel (see figure). All cells should be highlighted, while cellular debris should be excluded.

Imprecise cell count: I see large variation between technical replicates

The cell counting precision, often quantified as the coefficient of variation from replicate counts, is affected by many variables, including:

1. Cell concentration: A low cell sample concentration will negatively affect the counting precision. See our Technical note: Effects of sample concentration on cell counting variation NucleoCounter® NC-202™ *(document no. 994-2030)*
2. Liquid handling: The cell suspension should be thoroughly mixed before the sample is aspired into the Via2-Cassette™

3. Cell sample size: The Via2-Cassette™ can aspirate from 200 µl sample in a 1.5 ml tube, however increasing the sample volume improves the precision
4. Consistent protocol execution: Human variation and possibly error in sample handling causes variation between samples and replicas
5. Sample preparation: Ensure that cell sampling and sample dilutions are made using wide orifice tips to avoid 'bottleneck effects'

Inaccurate viability

We refer to the 'Microcarriers' protocol if the total cell population cannot be robustly stained with AO or a representative sample is not present inside the second cassette. The 'Microcarrier' protocol measures non-viable cells in the second cassette and calculates the viability using the total count from the first cassette. Examples of samples best analyzed with the 'Microcarriers' protocol include: Cells containing melatonin that quench the AO signal, cell samples containing lipid droplets that are stained by AO, cells grown in spheroids or cells grown on microcarriers.

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'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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