Application Note No. 2039
NucleoCounter® NC-202™

Leukocyte Cell Count & Viability from Blood Samples using the Via2-Cassette™

Product description

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

Application

This application note describes how to determine the cell concentration and viability of leukocytes in a blood sample, using the **Lysis 4** lysis buffer and the Via2-Cassette™. Treating a blood sample with **Lysis 4** causes rupture of erythrocytes. Lysing the erythrocytes causes an even distribution of hemoglobin throughout the sample, which in turn reduces quenching of the fluorescent signal.

The NucleoCounter® NC-202™ provides a simple and robust method to determine cell concentration and viability with the Via2-Cassette™ This application was developed for ordinary mammalian blood.

Introduction

The NucleoCounter® NC-202™ accurately counts cells using the Via2-Cassette™. Inside the cassette, cells are stained by two dyes, acridine orange (AO) and DAPI, which label the total and the non-viable cell populations, respectively. Once loaded, place the Via2-Cassette™ in the NucleoCounter® NC-202™ and press RUN to acquire data. The NC-View™ software automatically analyses and presents cell concentration and viability for fast and easy data acquisition.

Procedure

Optimal analysis requires ≥ 100 µl cell sample. Transfer a representative sample to a 1.5 ml microcentrifuge tube. Treat the sample with **Lysis 4** buffer to lyse the erythrocytes and increase the fluorescent signal, then load a sample into the Via2-Cassette™.

Materials needed
- Blood sample
- **Lysis 4**, room temperature (≥20°C)
- Via2-Cassette™

Procedure

Determine the total cell concentration from a cell sample mixed 1:10 with **Lysis 4**:
1. Pipette the cell suspension to obtain a homogenous suspension and transfer a representative sample to a microcentrifuge tube, then mix 1:10 with **Lysis 4** (e.g., add 180 µl of **Lysis 4** to 20 µl of cell suspension).
2. Incubate 3 min at room temperature (≥ 20°C)
3. Load a cell sample by inserting the tip of the Via2-Cassette™ into the mix and press the piston
4. Insert the Via2-Cassette™ into the NucleoCounter® NC-202™, select the 'Blood' protocol and press RUN

Within approximately 30 seconds, the following results are displayed: Total (cells/ml), Live (cells/ml), Dead (cells/ml), Viability (%), Aggregates (%; cell clumps (of 5 cells or more)), Dilution factor, and instrument 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 the sample to ensure accurate cell counting. Then count the diluted cell sample as described above. If possible, avoid using PBS or other saline solutions as diluent, it may alter the distribution of live and dead cells through unintended adhesion of living cells. We recommend dilution in the culture medium or in serum-free mediums, e.g. X-VIVO™ 15.
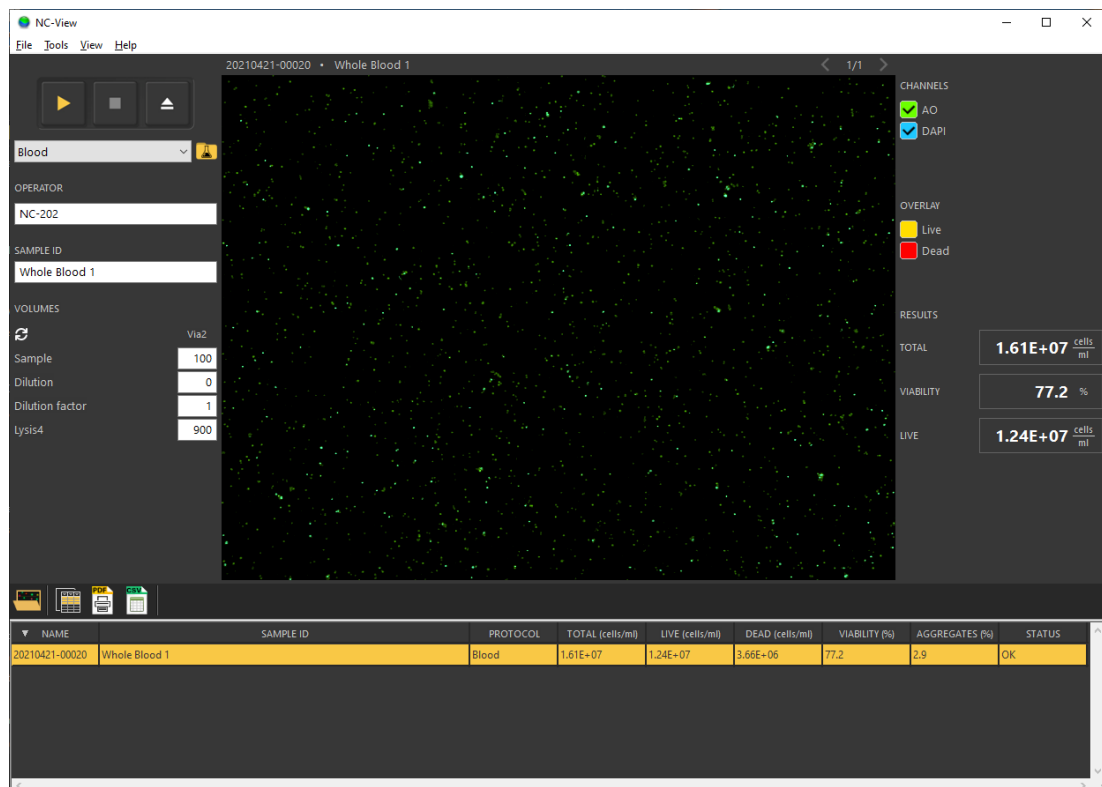
Viability

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 total concentration of cells (i.e. AO-positive cells)
C_{nv}: The concentration of non-viable cells (i.e. DAPI-positive cells)

Picture of NC-View™ software after running the Blood protocol on an untreated blood sample using the NucleoCounter® NC-202™. AO and DAPI channels are shown in green and blue, respectively. Enabling the image overlay will display live (yellow) and dead (red) cells, as identified by the NC-View™ software. Results are presented in the right-hand side panel and in the file list below.

Troubleshooting

Inaccurate cell count: My cell count is either too high or too low

When analyzing a new cell line, it is important to verify that the cells are correctly identified and recorded. Cells identified by the NC-View™ software can be shown by clicking cell overlay in the right-hand panel. 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 aspiring the sample into the Via**2**-Cassette™
3. Cell sample size: The Via**2**-Cassette™ can aspirate from a 200 µl total volume sample in a 1.5 ml tube; however, increasing the sample volume improves the precision
4. Consistent protocol execution: Human variation and possible errors in sample handling cause variations between samples and replicas
5. Sample preparation: Ensure that cell sampling and sample dilutions are made using wide orifice tips to avoid 'bottleneck effects'
6. Erythrocyte lysis is temperature dependent. Make sure that **Lysis 4** is $\geq 20°$ C

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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Document type: Application Note Document version: 1.6
Document no.: 994-2039 Approved by: ell Approved date: 25jul2022 System ID: 10024

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