Application Note No. 2039. Rev. 1.2
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 $5 \times 10^4$ - $1 \times 10^7$ cells/ml. If the cell concentration is above $1 \times 10^7$ 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:

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\% \text{ Viability} = \frac{C_t - C_{nv}}{C_t} \times 100\%
\]

% Viability: The percentage of viable cell in the cell sample  
C<sub>t</sub>: The total concentration of cells (i.e. AO-positive cells)  
C<sub>nv</sub>: 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:

2. Liquid handling: The cell suspension should be thoroughly mixed before aspiring the sample into the Via2-Cassette™
3. Cell sample size: The Via2-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 ≥ 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
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Product disclaimer
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