

## Application Note No. 2037. Rev. 1.2

# NucleoCounter® NC-202™

### Count & Viability of PBMCs - Via2-Cassette™

#### Product description

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

#### Application

This application note describes how to determine the viability and cell concentration of peripheral blood mononuclear cells (PBMCs) using the Via2-Cassette™. The Via2-Cassette™ provides a simple and robust method to determine cell concentration and viability with the NucleoCounter® NC-202™.

#### Introduction

Easily load a sample from a cell suspension into the Via2-Cassette™ tip by pressing the piston. Inside, 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.

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#### Procedure

For optimal analysis,  $\geq 200 \mu\text{l}$  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™.

#### Materials needed

- PBMC sample
- Via2-Cassette™

#### Procedure

1. Mix the cell suspension to homogeneity
2. Load a cell sample by inserting the tip of the Via2-Cassette™ in the cell suspension, then press the piston
3. Insert the loaded Via2-Cassette™ in the NucleoCounter® NC-202™, select the 'PBMC' protocol and press RUN

After approximately 30 seconds, the cell concentration and viability of the sample are displayed. The results available are: Total (cells/ml), Live (cells/ml), Dead (cells/ml), Viability (%), Aggregates (%; cell clumps of 5 cells or more), Diameter ( $\mu\text{m}$ ), DebrisIndex™, Dilution factor and 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 it with growth medium to ensure accurate cell counting. The diluted cell sample is then counted as described above. If possible, avoid using PBS or other saline solutions as diluent. We recommend dilution in cultivation medium or serum-free medium e.g. X-VIVO15. The Diameter ( $\mu\text{m}$ ) 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

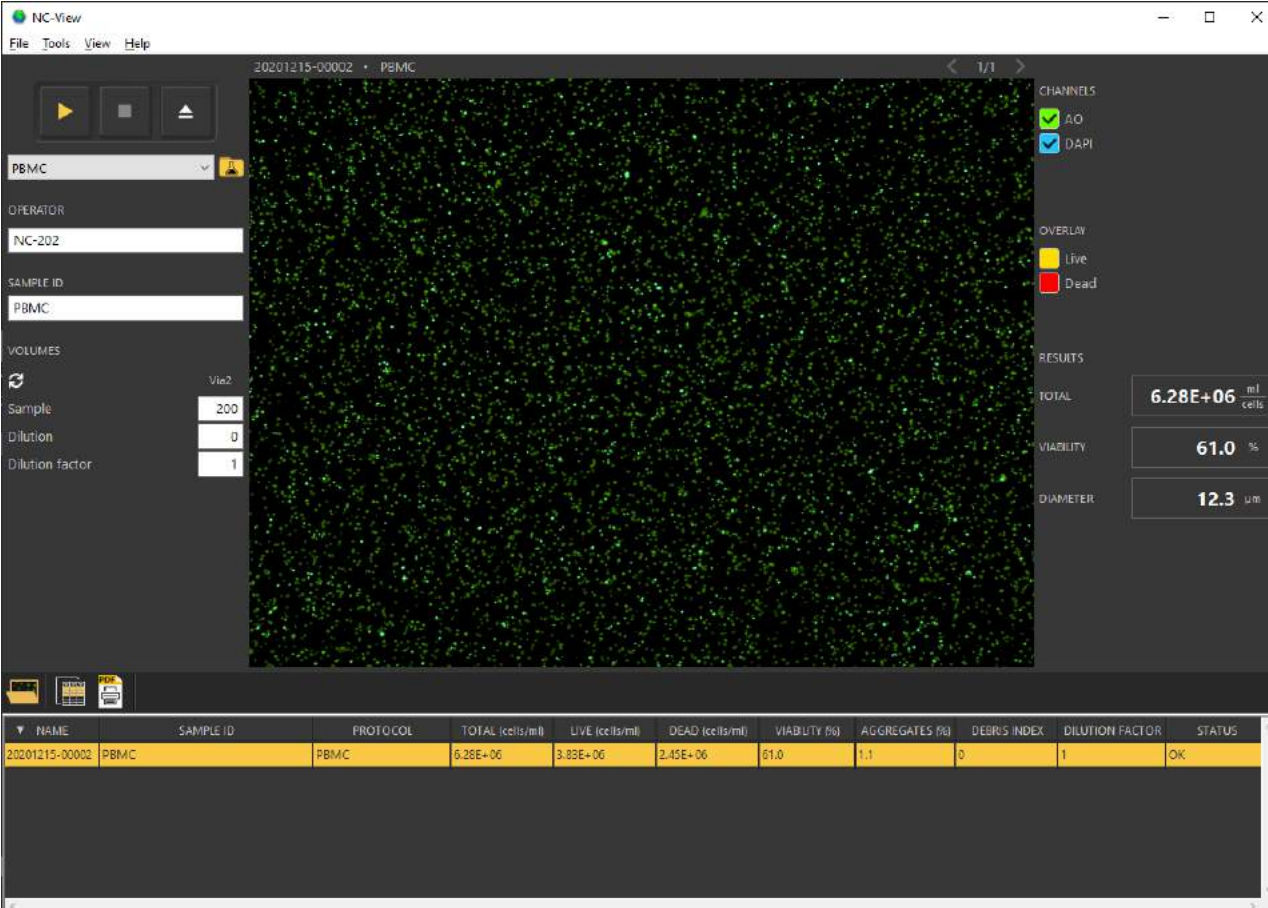
The viability percentage is calculated as follows:

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

**% Viability:** The percentage of viable cell in the cell sample

**$C_t$ :** The total concentration of cells (i.e. acridine orange positive cells)

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



NC-View

File Tools View Help

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PBMC

OPERATOR: NC-202

SAMPLE ID: PBMC

VOLUMES: View2

Sample: 200

Dilution: 0

Dilution factor: 1

CHANNELS:  AO,  DAPI

OVERLAY:  Live,  Dead

RESULTS: TOTAL: 6.28E+06 ml cells, VIABILITY: 61.0 %, DIAMETER: 12.3 μm

NAME	SAMPLE ID	PROTOCOL	TOTAL (cells/ml)	LIVE (cells/ml)	DEAD (cells/ml)	VIABILITY (%)	AGGREGATES (%)	DEBRIS INDEX	DILUTION FACTOR	STATUS
20201215-00002	PBMC	PBMC	6.28E+06	3.83E+06	2.45E+06	61.0	1.1	0	1	OK

Picture of NC-View™ software after running the PBMC protocol on a PBMC 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 live (yellow) and dead cells (red) 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 correctly identified and recorded. 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 aspirated 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 replicates
5. Sample preparation: Ensure that cell sampling and sample dilutions are made using wide orifice tips to avoid 'bottleneck effects'
6. The sample contains a high concentration of red blood cells. If the sample contains more than  $1 \times 10^6$  red blood cells/ml the hemoglobin of the red blood cells may quench the fluorescence and influence the results.

### 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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