

## Application Note No. 2026. Rev. 1.1

# NucleoCounter® NC-202™

### Count & Viability - 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™ provides a simple and robust method to determine cell viability and concentration with the NucleoCounter® NC-202™.

#### Introduction

A cell sample in suspension is easily loaded with the Via2-Cassette™ tip by pressing the piston. The cells are stained inside the Via2-Cassette™ by two dyes: Acridine Orange and DAPI that label the total and the non-viable cell population, respectively. Once loaded, place the Via2-Cassette™ in the NucleoCounter® NC-202™ and press RUN to acquire data. Viability and cell concentration data is automatically analyzed and presented.

---

#### Procedure

Adherent or semi-adherent cells should be released (e.g. by trypsin/EDTA treatment) from the cell culture surface. For optimal analysis  $\geq 200 \mu\text{l}$  cell sample is required. A representative sample should be transferred to a 1.5 ml micro centrifuge tube, which inner shape fits with the Via2-Cassette™ tip.

#### Materials needed

- Cell sample in suspension
  - Via2-Cassette™
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 'Count & Viability' protocol and press RUN.

In approximately 30 seconds 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 ( $\mu\text{m}$ ), Aggregates (%), Debris Index, Dilution factor and Status.

## 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.

## Viability

The percent viability is calculated from:

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

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

**C<sub>t</sub>:** The total concentration of cells (Acridine Orange positive cells)

**C<sub>nv</sub>:** The concentration of non-viable cells (DAPI positive cells)

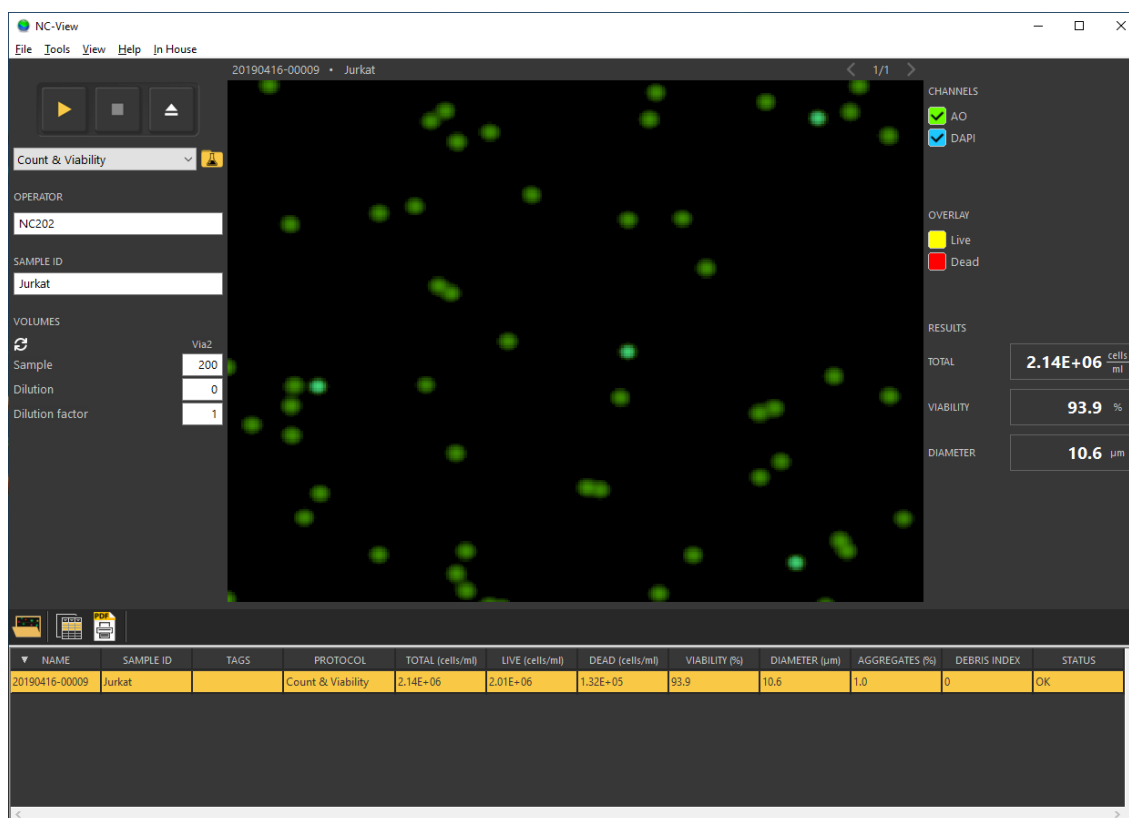


Figure 1: Cell count and viability of Jurkat cells using the NucleoCounter® NC-202™. The acquired image is shown central. Acridine Orange and DAPI stained cells appear green and blue, respectively. Enabling the image overlay displays the identified live (yellow) and dead cells (red). The counting results 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.

### 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'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.

### **Copyright**

Copyright © ChemoMetec A/S 2019. All rights reserved. No part of this publication and referred documents may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior written consent of ChemoMetec A/S, Gydevang 43, DK-3450 Allerød, Denmark.

ChemoMetec and NucleoCounter are registered trademarks owned by ChemoMetec A/S. Via2-Cassette and NC-View are trademarks of ChemoMetec A/S. All other trademarks are the property of their respective owners.