Application Note No. 2034

NucleoCounter® NC-202™

Count & Viability of Cells Grown on Microcarriers - 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 a variety of cells grown on microcarriers using the Via2-Cassette™ and **Lysis 1** with the NucleoCounter® NC-202™. Treatment of cell samples with **Lysis 1** facilitates detachment from microcarriers, disaggregation, and cell lysis resulting in a suspension of released single nuclei. The Via2-Cassette™ provides a simple and robust method to determine cell concentration and viability with the NucleoCounter® NC-202™ instrument.

Introduction

Two Via2-Cassettes™ are required to determine cell count and viability for this application. To measure the total cell concentration, mix the microcarrier sample in suspension with **Lysis 1** and load into the first Via2-Cassette™, thus detaching cells from the microcarriers and staining the cell nuclei with DAPI. Determine the cell concentration of dead cells with a second Via2-Cassette™, which stains the non-viable cell population with DAPI. 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.

Procedure

Treating a microcarrier culture with **Lysis 1** buffer detaches cells from the microcarrier by lysis of the cell membrane, in turn producing a suspension of released single nuclei. For optimal analysis, ≥ 1000 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™. The microcarrier size may compromise the distribution of the sample between tubes and may be avoided by using a wide orifice pipette tip or manually cutting the pipette tip to avoid clogging.

Materials needed

- Microcarrier cell sample in suspension
- **Lysis 1**
- Via2-Cassette™

Procedure

1. Stir the microcarrier-cell culture vessel to obtain a homogenous cell sample and draw two samples of e.g. 500 µl into 1.5 ml microcentrifuge tubes

2. Determine the total cell concentration from a cell sample mixed 1:1 with **Lysis 1**:
   a. Pipette the microcarrier cell suspension in the culture vessel to obtain a homogenous cell sample then mix 1:1 with **Lysis 1** (e.g., add 500 µl of **Lysis 1** to 500 µl of cell suspension)
   b. Mix by thoroughly by pipetting

c. (Optional) Incubate at room temperature to ensure release from the microcarriers. Usually, 3 minutes incubation is sufficient. However, this should be optimized for each sample type
d. Allow microcarriers to settle until they form a clear pellet
e. Load by inserting the tip of the first Via2-Cassette™ without disturbing the pellet and press the piston
f. Insert the Via2-Cassette™ into the NucleoCounter® NC-202™, select the 'Microcarriers' protocol and press RUN

3. Determine the non-viable cell concentration from an untreated cell sample:
   a. Mix the cell suspension (**without Lysis 1**-treatment) to obtain a homogenous suspension
   b. Allow microcarriers to settle until they form a clear pellet
   c. Insert the tip of the second Via2-Cassette™ into the cell suspension without disturbing the pellet and press the piston
   d. 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, then select 'Cancel' after analysis of the first Via2-Cassette™, whereby only the total cell concentration will be provided.

Within one minute, the viability and cell concentration of the sample are displayed. The available results are: Total (cells/ml), Live (cells/ml), Dead (cells/ml), Viability (%), Dilution factor and Status.

Notes

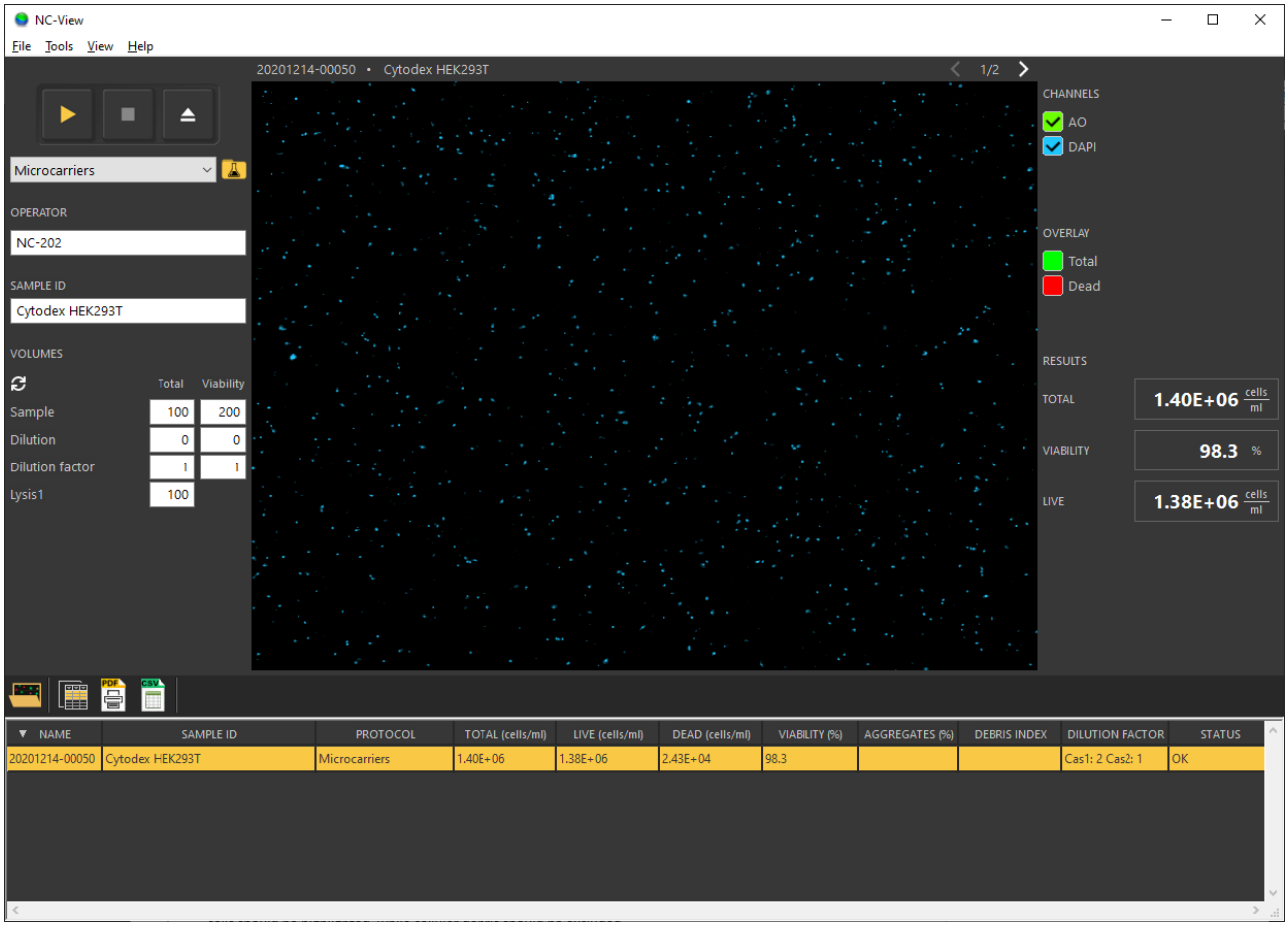
To ensure robust and reliable results, the cell suspension concentration should be in the range of 5×10^4 - 1×10^7 cells/ml. If the cell concentration is above 1×10^7 cells/ml, dilute it with growth media to ensure accurate cell counting. The diluted cell sample is then counted as described above. **Lysis 1** will precipitate below 15°C. Check buffer for precipitation before use. Re-dissolve any precipitation by warming to room temperature.

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 (DAPI positive cells in the first Via2-Cassette™)
C_{nv}: The concentration of non-viable cells (DAPI positive cells in the second Via2-Cassette™)



Picture of NC-View™ software after running the Microcarriers protocol on a HEK293T cell sample grown on Cytodex 1 microcarriers using the NucleoCounter® NC-202™. The AO and DAPI channels are shown in green and blue, respectively. Enabling the image overlay displays total (green) in view 1 and dead cells in view 2 (red) identified by the software. Counting results are presented in the right panel and in the file list below.

Troubleshooting

Viability and live cell count are negative:

If the concentration of dead cells is more than 10% higher than the total cell concentration, a warning will be given in the status column. This result can be due to inaccuracies when diluting the cell sample or because the user did not correctly update the volume fields before starting the protocol.

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 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'

Optimizing sampling

The sampling procedure may greatly influence the accuracy and precision of the cell count. Thus, to eliminate potential error-sources ensure that: a) A consistent mixing of the culture vessel before sampling. b) Use a wide orifice / wide bore tip to ensure easy passage of microcarriers, which otherwise may clog the tip, creating a filter-effect. Avoid regular p200 tips. c) Plastic sticking effects. Microcarriers often stick to plastic resulting in uneven distributions of cells in the sample tube, therefore, mix by pipetting and avoid inverting the tube. d) Avoid small volumes. If possible, use large volume e.g. 1 ml of 10 ml for greater consistency.

Optimizing incubation time

Specific microcarrier and cell types can form strong interactions that brief exposure to cannot break. Prolonged incubation may release more cells. However, nuclei are not stable in **Lysis 1**, and the cell count may therefore decline if the incubation time becomes too long.

Setup a time course experiment and determine the time required for maximum release of cells. For example, incubate for 0, 2, 4, 6, 8, 10 minutes in **Lysis 1**. You can also use a microscope to verify that cells are released from the microcarriers, if they still adhere, increase the incubation time.

Settling microcarriers

Microcarriers usually settle very fast; this is visible to the naked eye: the liquid clears, and a pellet forms. Settling time depends on the specific microcarriers. Cells, detached from the microcarriers settles more slowly, as they are much less dense than microcarriers. Once microcarriers have settled, insert the Via2-Cassette™ tip into the middle of the solution and press the plunger. Ensure that the sample is drawn at similar position every time. Do not disturb the pellet, as microcarriers can enter the Via2-Cassette™ and risk clogging the flow channels.

Releasing cells by pipetting

Mix the solution thoroughly by pipetting. Be consistent in your pipetting, e.g. 10 times up and down. It is also possible to use a Pasteur pipette to provide a more consistent shearing force due to the long and narrow tip. Avoid creating foam, do not vortex or invert the tube, this will introduce errors. When inverting the tube, microcarriers will stick to the lid and foam.

High variance or low viability counts

Check by bright field microscopy that the cells are attached to the microcarriers: Overgrown microcarriers and clumping microcarriers are difficult to pipette and representatively sample. This results in high variance and low reproducibility.

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.