Technical note No. 0221 Rev. 1.1

NucleoCounter® NC-200™

Viability and Cell Count of Microcarrier Cultured Cells using Via-1 Cassettes with Reagent A100 and Reagent B

Product description

The NucleoCounter® NC-200™ system enables the user to perform automated cell counting and analyses on a broad range of eukaryotic cells.

Application

The Via1-Cassette™, **Reagent A100** and **Reagent B** is used with the NucleoCounter® NC-200™ to determine the concentration of cells grown in microcarrier suspension cultures. **Reagent A100** is added to the culture, releasing the cells from the microcarriers and bringing the cell nuclei into suspension. **Reagent B** stabilizes the nuclei before analysis. Cells are loaded into the Via1-Cassette™ and are counted in the NC-200™ instrument.

Introduction

When adherent anchorage-dependent cells are cultured on microcarriers, large clusters of beads and cells are formed. Enzymatic digestion of the cell-bead complex is typically required to allow the cells to be counted. This is a time-consuming procedure and cells are often not fully released from the microcarriers, and are forming aggregates, which leads to an imprecise cell count.

The NC-200™ allows fast and precise quantification of the total cell count and viability of cells, grown on microcarriers without the need for enzymatic digestion.

Procedure

The microcarrier suspension culture is treated with **Reagent A100** lysis buffer. The lysis buffer permeabilizes the cell plasma membranes and releases the nuclei from the solid support. The nuclei are then stabilized with **Reagent B**. The cell sample, diluted 1:1:1 with **Reagent A100** and **Reagent B,** is drawn into a Via1-Cassette™. The fluidic channels of the Via1-Cassette™ contain DAPI that immediately stains DNA, rendering the nuclei fluorescent bright blue. The loaded Via-1 Cassette™ is inserted in the NC-200™ instrument that automatically acquires a blue fluorescent image, identifies the nuclei through image analysis and calculates the total cell count. In the second step, the dead cell count is obtained by loading the untreated microcarrier suspension culture into a Via1-Cassette™. In the absence of **Reagent A100** and **Reagent B** only the dead cells unattached to the microcarriers will be stained blue. The NC-200™ software automatically calculates the total cell concentration and the viability.

<u>Materials needed</u>

Microcarrier cell suspension culture to be counted
Two Via1-Cassette™ units (Cat. #941-0012)
Reagent A100 (Cat. #910-0003)
Reagent B (Cat. #910-0002)

1. The first step is to determine the total cell concentration of the microcarrier cell sample.
    a. Stir the microcarrier-cell culture vessel to obtain a homogenous cell sample and transfer to a separate vial. Then add one volume of **Reagent A100**. For example, to 100 µl of microcarrier cell suspension add 100 µl of **Reagent A100**. Mix by pipetting.

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b. Add one volume of **Reagent B** to the mixture of the microcarrier cell suspension and **Reagent A100.** For example, to 200 µl of the mixture of microcarrier cell suspension and **Reagent A100**, add 100 µl of **Reagent B**. Mix by pipetting.
c. To avoid clogging of the Via1-Cassette™, leave the microcarrier cell sample diluted 1:1:1 with the **Reagent A100** and the **Reagent B** in a rack for 1-2 min until the microcarriers have settled in the bottom of the tube.
d. When the microcarriers have settled to the bottom of the tube, insert the Via1-Cassette™ halfway into the liquid and press the piston to collect a sample (Figure 1).
e. Select the **"Viability and Cell Count – A100 and B Assay"** and press RUN. Insert the loaded Via1-Cassette™ containing the sample diluted 1:1:1 with **Reagent A100** and **Reagent B** into the NucleoCounter® NC-200™ and click "OK'.

2. The second step is to determine the concentration of non-viable cells in the undiluted cell sample.
a. Mix the undiluted microcarrier cell suspension to obtain a homogenous suspension by pipetting and transfer at least 100 µl to a separate vial (without the **Reagent A100** and **Reagent B** treatment).
b. To avoid clogging of the Via1-Cassette™, incubate the undiluted microcarrier cell sample in a rack for 1-2 min.
c. When the microcarriers have settled to the bottom of the tube, insert the Via1-Casstte™ halfway into the liquid and press the piston to collect a sample (Figure 1).
d. When prompted by a message box, replace the first Via1-Cassette™ with the second Via1-Cassette™ loaded with the undiluted cell suspension and click "OK".

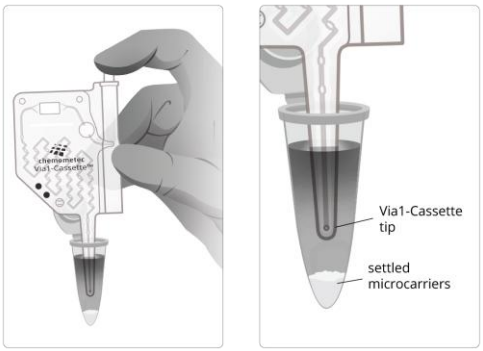

Figure 1. Correct loading of the Via1-Cassette™. To avoid microcarriers clogging the Via1-Cassette™, the cell sample is incubated for 1 to 2 minutes in a rack, allowing the microcarriers to settle down to the bottom of the tube. After the incubation time, the Via1-Cassette™ is inserted halfway into the liquid and the cell sample is collected by pressing the piston of the Via1-Cassette™.

Viability

The NucleoView software calculates the viability as follows:

$$\%viability = \frac{C_{total} - C_{nv}}{C_{total}} \cdot 100\%$$

C_{total} The concentration of total cells. Via1-Cassette™ loaded with cell sample diluted 1:1:1 with **Reagent A100** and **Reagent B**.

C_{nv} The concentration of non-viable cells. Via1-Cassette™ loaded with undiluted cell sample without the **Reagent A100** and **Reagent B**.

Notes:

Cell Counting without Viability:
Purpose: Determine the total cell concentration.

Procedure: Select the **"Count of Aggregated Cells - A100 and B Assay"** and follow step 1a to 1e in the protocol above.

Evaluate the need for extended A100 incubation:
Purpose: Determine the **Reagent A100** incubation time needed to maximize the release of nuclei from the microcarrier support. Specific microcarrier and cell types can form strong interactions that brief exposure to **Reagent A100** cannot break. Extending the incubation of the microcarrier cell suspension in **Reagent A100** before adding **Reagent B** may increase the number of released nuclei. However, nuclei are not stable in **Reagent A100** and the cell count may decline over time as the nuclei are disrupted.

Procedure: Perform a time course measurement where the microcarrier cell suspension is incubated in **Reagent A100** in 1 minute increments for up to 10 minutes before stabilizing the sample with **Reagent B** to establish the time point where nuclei are most effectively released. Use triplicates for each time point.

Anticipated Results: Figure 2 is an example of culture conditions where all nuclei are immediately released from the microcarriers. No extended incubation is necessary. Figure 3 is an example where a 4 to 6 minutes incubation time in **Reagent A100** is necessary to achieve maximal release of nuclei. In this latter case, include a 5 minutes incubation time in **Reagent A100** in step 1a of the protocol above.

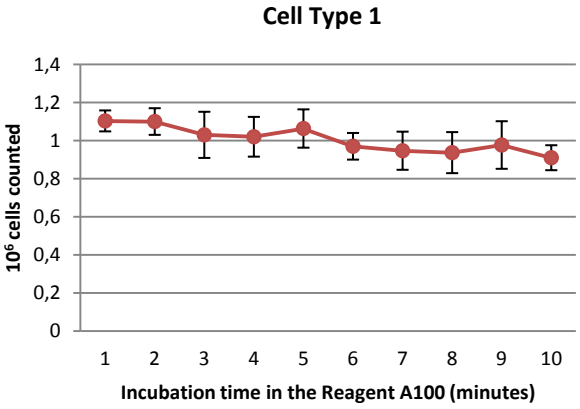

Figure 2. Cell count slowly declines with extended incubation in **Reagent A100** before addition of **Reagent B** and sample loading. Each data point corresponds to 3 measurements. The error bars show the standard deviation.

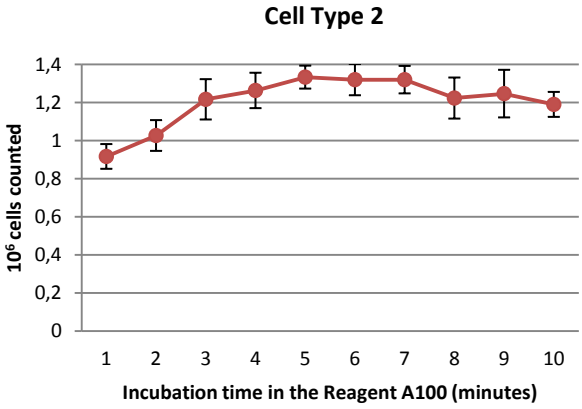

Figure 3. Cell count is noticeably increased when incubation in **Reagent A100** exceeds 2 minutes. Each data point corresponds to 3 measurements. The error bars show the standard deviation.

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-200™ user's 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-200™ system is FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE. The results presented by the NucleoCounter® NC-200™ system depend on correct use of the reagents, cassettes and the NucleoCounter® NC-200™ instrument and may depend on the type of cells being analyzed. Refer to the NucleoCounter® NC-200™ 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

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