Technical note No. 0221 Rev. 2.0

NucleoCounter® NC-200™

Viability and Cell Count of Microcarrier Cultured Cells using Via1-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 a culture sample, 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 NucleoCounter® 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; cells are often not fully released from the microcarriers and are forming aggregates, leading 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.

Experimental Procedure
The microcarrier suspension culture sample is treated with **Reagent A100** lysis buffer, which permeabilizes the cell plasma membranes and releases the cells 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 channel in the Via1-Cassette™ contains DAPI that immediately stains DNA, rendering the nuclei fluorescent bright blue. The loaded Via1-Cassette™ is inserted in the NucleoCounter® 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 dead cells detached from microcarriers are stained. The NucleoView NC-200™ software automatically calculates the total cell concentration and the viability.

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

1. Total cell concentration.

   a. Stir the microcarrier-cell culture vessel to obtain a homogenous cell sample. (See Note 1)
   b. Sample 300 µL into a 1.5 mL microcentrifuge tube.
   c. Add 300 µl **Reagent A100**.
   d. Mix by thoroughly by pipetting.
   e. (Optional) Incubate at room temperature. (See note 2)
   f. Add 300 µl **Reagent B.**

g. Mix by pipetting.
h. Allow microcarriers to settle until the solution clears and a pellet forms (approx. 20 sec. settling time depend on microcarrier type. See note 3).
i. Insert a Via1-Cassette™ halfway into the liquid, and press the piston to collect a sample (Figure 1A).
j. Select the **"Viability and Cell Count – A100 and B Assay"**.
k. Insert the loaded Via1-Cassette™ into the NucleoCounter® NC-200™ and press "RUN'. While, the analysis is running, prepare the sample for analysing the non-viable cell concentration.

2. Non-viable cell concentration.

a. Stir the microcarrier-cell culture vessel to obtain a homogenous cell sample
b. Sample 300 µL into a 1.5 mL microcentrifuge tube.
c. Mix by pipetting. (See Note 4)
l. Allow microcarriers to settle until the solution clears and a pellet forms (around 20 sec. settling time depend on microcarrier type. See Note 3).
d. Insert the Via1-Casstte™ halfway into the liquid, and press the piston to collect a sample (Figure 1A).
e. When prompted by a message box in the NucleoView NC-200™ software, replace the first Via1-Cassette™ with the second Via1-Cassette™ loaded with the undiluted cell suspension and click "OK".

Viability

The NucleoView NC-200™ software calculates the viability as follows:

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

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 **Reagent A100** and **Reagent B**.

Notes:

1. Sampling from microcarrier-cell cultures

The sampling procedure may greatly influence the accuracy and precision of the cell count. Thus, to eliminate potential error-sources ensure: 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.

2. Optimizing **Reagent A100** incubation

Specific microcarrier and cell types can form strong interactions that brief exposure to **Reagent A100** cannot break. Prolonged **Reagent A100** incubation may release more cells, however nuclei are not stable in **Reagent A100,** and the cell count may decline over time.

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 min in **Reagent A100** before adding **Reagent B**. Measure the released cells with the "**Count of Aggregated Cells – A100 and B Assay**". Expect that the concentration increases with time until it peaks (Figure 1B). Usually 2-4 min incubation is sufficient. Use a microscope to verify that cells are released from the microcarriers, if they still adhere, increase the incubation time.

3. Settling microcarriers

The microcarriers settle in approximately 20 sec; this is visible to the naked eye: the liquid clears, and a sediment forms. Settling time depends on the specific microcarriers. Cells, detached from the microcarriers are still in the supernatant, as they are much less dense than microcarriers. Once microcarriers have settled, insert the Via1-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 sediment**, as microcarriers can enter the Via1-Cassette™ and risk clogging the flow channels.

4. 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,** so do not vortex or invert the tube, this will introduce errors. When inverting the tube, microcarriers will stick to the lid and foam.

5. 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.

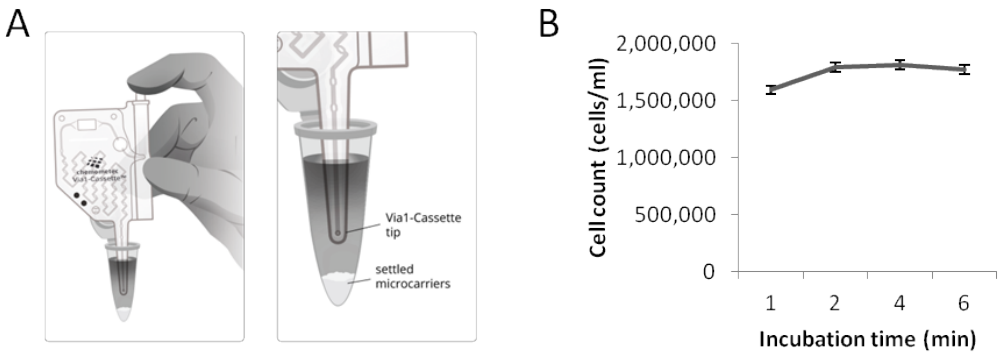

Figure 1. (a) Correct loading of the Via1-Cassette™, (b) an example of **Reagent A100** incubation optimization.

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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