Technical note No. 994-3815 Rev. 1.0

NucleoCounter® NC-250™

Viability and Cell Count of Microcarrier Cultured Cells

Product description
The NucleoCounter® NC-250™ system enables the users to perform automated cell counting and analyses on a broad range of eukaryotic cells.

Application
The NC-Slide A2™, **Solution 12**, **Reagent A100** and **Reagent B** is used with the NucleoCounter® NC-250™ to determine the concentration and viability 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. At the same time **Solution 12** enables staining of all cells with DAPI. **Reagent B** stabilizes the nuclei before analysis. The sample is loaded into an NC-Slide A2™ and inserted into the NucleoCounter® NC-250™ where viability and cell concentration is automatically determined.

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. However, this is a time-consuming procedure and often cells are not fully released from the microcarriers. As a result, these cell aggregates will lead to an imprecise cell count. The NC-250™ 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 and **Solution 12** which contains DAPI. The lysis buffer permeabilizes the cell plasma membranes, releases the nuclei from the solid support and the nuclei are instantly stained with DAPI. The nuclei are then stabilized with **Reagent B**. The microcarrier cell sample, diluted with **Solution 12**, **Reagent A100**, and **Reagent B** is loaded into the first chamber a NC-Slide A2™ to get the total cell count. The second chamber is loaded with the microcarrier cell sample pre-treated with **Solution 12,** only staining the dead cells unattached to the microcarriers. The loaded NC-Slide A2™ is inserted into the NC-250™ instrument that use fluorescent imaging to identify the nuclei in each chamber to acquire the total cell count and non-viable count respectively. The viability is calculated from these two numbers.

Materials needed
Microcarrier cell suspension culture to be counted
NC-Slides A2™ (Cat. #942-0001)
Solution 12 (500 µg/ml DAPI, Cat. #910-3012)
Reagent A100 (Lysis buffer, Cat. #910-0003)
Reagent B (Stabilizing buffer, Cat. #910-0002)

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1. The first step is to determine the total cell concentration of the microcarrier cell sample.

a. Add 1 volume of **Solution 12** into 99 volumes of **Reagent A100**. For example, add 1 µl of **Solution 12** into 99 µl of **Reagent A100**. Note: do not store this mixture, but prepare a new each time the assay is performed.

b. Stir the microcarrier-cell culture vessel to obtain a homogenous cell sample and transfer a sample volume to a separate 1.5 ml microcentrifuge tube. Then add one volume of the mixture of **Solution 12** and **Reagent A100**. For example, to 100 µl of microcarrier cell suspension add 100 µl of the mixture of **Solution 12** and **Reagent A100**. Mix by pipetting (if using macroporous microcarriers see notes section).

c. Add one volume of **Reagent B** to the mixture of the microcarrier cell suspension, **Solution 12** and **Reagent A100**. For example, to 200 µl of the mixture of microcarrier cell suspension, **Solution 12** and **Reagent A100**, add 100 µl of **Reagent B**. Mix by pipetting.

d. To avoid clogging of the NC-Slide A2™, leave the microcarrier cell sample diluted with the **Solution 12**, **Reagent A100** and the **Reagent B** in a rack for 1-2 minutes until the microcarriers have settled to the bottom of the tube. When the microcarriers have settled to the bottom of the tube, insert the pipette halfway into the liquid and collect a sample (Figure 1). Load ~30 µl of the sample for the total count into the first chamber of the NC-Slide A2™.

2. The second step is to determine the concentration of non-viable cells in the microcarrier cell sample.

a. Stir the microcarrier-cell culture vessel to obtain a homogenous cell sample and transfer at least 198 µl to a separate 1.5 ml microcentrifuge tube (without the **Reagent A100** and **Reagent B** treatment). Then add 1 volume of **Solution 12** into 99 volumes of the microcarrier cell sample. For example, add 2 µl of **Solution 12** into 198 µl of the microcarrier cell sample. Mix by pipetting.

b. To avoid clogging of the NC-Slide A2™, leave the microcarrier cell sample diluted with the **Solution 12** in a rack for 1-2 minutes until the microcarriers have settled to the bottom of the tube. When the microcarriers have settled to the bottom of the tube, insert the pipette halfway into the liquid and collect a sample (Figure 1). Load ~30 µl of the sample for the dead count into the second chamber of the NC-Slide A2™.

c. Select the **"Viability and Cell Count – A100 and B Assay"** and sample unit **NC-Slide A2™**. Insert the loaded NC-Slide A2™ containing the samples for the total count and the dead count onto the tray of the NucleoCounter® NC-250™ and click "RUN'. The NC-Slide™ is for one-time-use only.

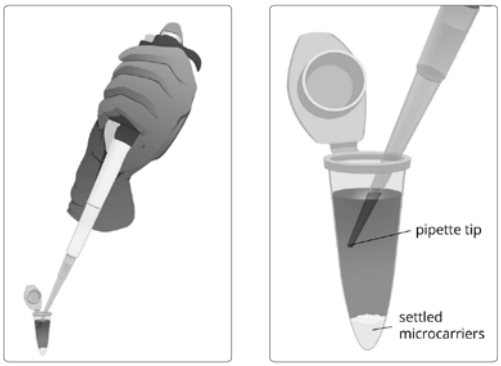

Figure 1. Correct pipetting of the microcarrier cell suspension. To avoid microcarriers clogging the NC-Slide A2™, the microcarrier cell sample is incubated for 1 to 2 minutes in a rack, allowing the microcarriers to settle down to the bottom of the tube. After the incubation time, the pipette is inserted halfway into the liquid and the sample is collected.

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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. The NC-Slide A2™ loaded with cell sample diluted with **Solution 12**, **Reagent A100** and **Reagent B**.
C_{nv} The concentration of non-viable cells. The NC-Slide A2™ loaded with cell sample diluted with **Solution 12**.

Notes:

Cell Counting without Viability:
Purpose: Count the total amount of cells.

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

Evaluate Reagent A100 incubation time for macroporous microcarriers:
Purpose: Determine whether extended incubation in **Reagent A100** is needed to achieve complete release of nuclei from the microcarrier support.
Some culture conditions (typically macroporous microcarriers) create conditions where brief exposure to **Reagent A100** is not sufficient to fully release the nuclei from the microcarrier support. Extending the **Reagent A100** incubation in step 1a will promote release of tightly bound nuclei. Nuclei, however, are not stable in **Reagent A100** so the ideal incubation time should be determined experimentally.

Procedure: Perform a time course experiment where the microcarrier-cell suspension is incubated in **Reagent A100** for 0, 2, 4, 6, 8 and 10 minutes before stabilizing the sample with **Reagent B**, to establish the time point where nuclei are most effectively released. Consistent pipette mixing is important to reduce variation. Use triplicate readings using the **"Count of Aggregated Cells - A100 and B Assay"** for each time point.

Anticipated Results: If the total cell count is not significantly increased from 0 to 4 min incubation, extended **Reagent A100** is not necessary, and the standard protocol should be used. If the total cell count noticeably increases over time, the optimal incubation time should be determined and incorporated into the microcarrier counting procedure.

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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-250™ 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-250™ system is FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE. The results presented by the NucleoCounter® NC-250™ system depend on correct use of the reagents, cassettes and the NucleoCounter® NC-250™ instrument and may depend on the type of cells being analyzed. Refer to the NucleoCounter® NC-250™ 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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