Viability and Cell Count of Microcarrier Cultured Cells using Via-1 Cassettes

Product description
The NucleoCounter® NC-3000™ system enables the user to perform automated cell counting and analyses on a broad range of eukaryotic cells.

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-3000™ allows fast and precise quantification of the total cell count and viability of cells, grown on microcarriers without the need for enzymatic digestion.

Application
The Via1-Cassette™, Reagent A100 and Reagent B is used with the NucleoCounter® NC-3000™ 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-3000™ instrument.

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-3000™ 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. The NC-3000™ software automatically calculates the total cell concentration and the viability.

Materials needed
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 a sample volume to a separate 1.5 ml microcentrifuge tube. 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 (if using macroporous microcarriers see notes section).
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 minutes 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 onto the tray of the NucleoCounter® NC-3000™ 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 200 µl to a separate 1.5 ml microcentrifuge tube (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 minutes.

c. 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).

d. When prompted by a message box in Nucleoview™, 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:

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\text{%viability} = \frac{C_{\text{total}} - C_{\text{nv}}}{C_{\text{total}}} \times 100\%
\]

- \(C_{\text{total}}\) The concentration of total cells. Via1-Cassette™ loaded with cell sample diluted 1:1:1 with Reagent A100 and Reagent B.
- \(C_{\text{nv}}\) The concentration of non-viable cells. Via1-Cassette™ loaded with undiluted cell sample without 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 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.
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-3000™ 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-3000™ system is FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE. The results presented by the NucleoCounter® NC-3000™ system depend on correct use of the reagents, cassettes and the NucleoCounter® NC-3000™ instrument and may depend on the type of cells being analyzed. Refer to the NucleoCounter® NC-3000™ user’s guide for instructions and limitations.

Liability disclaimer
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