Application note No. 203

NucleoCounter® NC-200™

Viability and Cell Count using the Via1-Cassette™ - Aggregated Mammalian Cells

Product description

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

Application

The Via1-Cassette™ and Solution 10 used together with the NucleoCounter® NC-200™ can be used for determination of the viability and cell concentration of aggregating cells. Treatment of cell samples with Solution 10 facilitates disaggregation of cell aggregates resulting in single cell suspensions.

Introduction

In order to determine the cell concentration, a sample containing cells in suspension is diluted with Solution 10 (lysis buffer) and loaded into the Via1-Cassette™. The inside of the Via1-Cassette™ is coated with DAPI, which after lysis with Solution 10 stains all cell nuclei in the sample. When the cell sample is not treated with Solution 10 the dye only stains the non-viable cells. The volume of each Via1-Cassette™ has been calibrated to give a high precision of the resulting count.
The Via1-Cassette™ is placed in the NucleoCounter® NC-200™ where cell concentration is determined.

Procedures

If the cell line to be investigated is adherent or semi-adherent, then start by getting all cells into suspension using the preferred method of your laboratory (e.g. trypsin/EDTA treatment).

Materials needed
- Cells to be counted
- Solution 10
- Two Via1-Cassettes™

1. The first step is to determine the total cell concentration of a cell sample that is lysed with Solution 10.
    a. The cell suspension is mixed to obtain a homogenous suspension and a sample is mixed 1:1 with Solution 10. E.g., to 100 µl of cell suspension add 100 µl of Solution 10. Mix by pipetting. Load a cell sample by inserting the tip of the first Via1-Cassette™ into the diluted cell suspension and pressing the piston.
    b. Select the "**Viability and Cell Count - Aggregated Cells Assay**" and press RUN. Select "**OK**" when the loaded Via1-Cassette™ containing the sample diluted 1:1 with Solution 10 is in the NucleoCounter® NC-200™.
2. The second step is to determine the concentration of non-viable cells of an undiluted cell sample.
    a. The cell suspension (**without** Solution 10 treatment) is mixed again to obtain a homogenous suspension. Draw a cell sample by inserting the tip of the second Via1-Cassette™ into the cell suspension and pressing the piston.
    b. When the message box requests it, replace the first Via1-Cassette™ with the second Via1-Cassette™ loaded with the cell suspension without addition of Solution 10 and select "**OK**".

After approximately 2 minutes the cell concentration (cells/ml) of the total cell count and the viability are displayed together with the cell diameter. The displayed cell concentration of the total cell count has been compensated for the dilution caused by the addition of Solution 10. If the cell sample has been further diluted or concentrated and the user has entered the volumes into the user interface the dilution factor has also been taken into account and the returned cell concentration is for the original cell concentration.

Note

To assure reliable results, it is recommended that the cell concentration of the counted cell suspension should be in the range of $5 \cdot 10^4$ cells/ml to $5 \cdot 10^6$ cells/ml. If the cell concentration is above $5 \cdot 10^6$ cells/ml, the cell suspension can be diluted with growth media or PBS to achieve the desired concentration. The diluted cell sample is then treated as described above. By inserting the value for the dilution volume in the dilution field on the user interface the returned cell concentration is for the original cell sample.

If the concentration of cells is below $5 \cdot 10^4$ cells/ml then the cell concentration may be increased by centrifugation followed by resuspension of the pellet using growth media or PBS. The resuspended cell sample is then treated as described in the procedure. By inserting a negative value representing the volume removed from the sample in the dilution field on the user interface the returned cell concentration is for the original cell sample.

Viability

The viability is calculated as follows:

$$\% \text{ viability} = \frac{Ct - Cnv}{Ct} \cdot 100\%$$
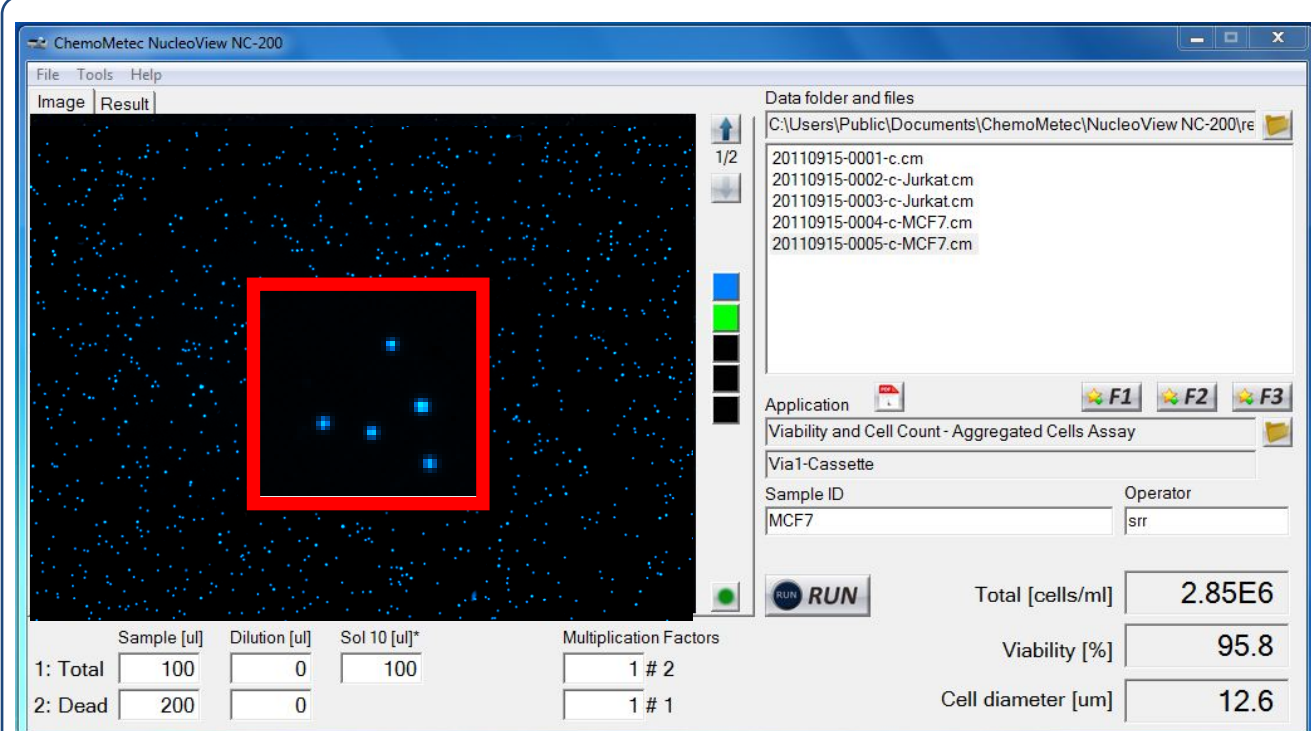
% viability	The percentage of viable cells in the cell suspension
C_t	The total concentration of cells
C_{nv}	The concentration of non-viable cells

Determination of cell concentration of aggregated MCF7 cells. The cells for the first Via1-Cassette™ were disaggregated by adding **Solution 10** and the cells for the second Via1-Cassette™ were untreated. The two samples were analyzed using the Viability and Cell Count - Aggregated Cells Assay. The total cell population is stained with DAPI and appears blue. An insert shows a close up of parts of the images. The results are presented at the bottom right and extended results are presented in the result tab page.

Troubleshooting

Inaccurate and imprecise counting:
When setting up a new cell line it is important to inspect that the cell line is counted correctly. The cells included in the total count can be marked by clicking on the overlay button in the bottom right corner of the image. Visual inspect the image to evaluate in the vast majority of the cells has been counted correctly. If this is not the case right click on the image file in question and choose "Show Counting Gates in Plot Manager". Inspect the gates displayed in the Plot Manager. If the gating is inappropriate adapt the gate(s) to cover the cell population (do not include debris and very large objects) using the Protocol Adaptation Wizard and save the changes to a new protocol. Note that the user is responsible for defining appropriate gating of the particular cell line.

Warning that the cell concentration of non viable cells is higher than the total cell concentration:
Make sure the problem is not due to interchanged samples of the total count sample treated with **Solution 10** and the non-viable count sample without treatment with **Solution 10**. Make sure that correct volumes for sample, dilution and **Solution 10** have been entered into the user interface.

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 might 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
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.

Document type: Application Note
Document no.: 994-0203

Document version: 1.5
Approved by: ell

Approved date: 22jul2022

System ID: 10525

ChemoMetec A/S · Gydevang 43 · DK-3450 Allerod · Denmark · support@chemometec.com · www.chemometec.com

Page 3 of 3