Application note No. 3016

NucleoCounter® NC-3000™

Counting of Aggregated Mammalian Cells using NC-Slides

Product description

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

Application

The NC-Slides, **Solution 10** and **Solution 12** used together with the NucleoCounter® NC-3000™ facilitates determination of the cell concentration of aggregating cell lines. The NC-Slide A2™ enables measurements of 2 cell samples at the same time with a high degree of precision, whereas the NC-Slide A8™ enables measurements of up to 8 samples at the same time with a moderate precision. The NC-Slide is for one-time-use only, and we strongly recommend discarding the slide after use even in cases where not all chambers have been used. An NC-slide with either two or eight samples is analyzed in approximately 90 seconds

Introduction

In order to determine the cell concentration of cell lines, a sample containing cells in suspension is mixed with **Solution 10** (lysis buffer) and **Solution 12** and loaded into a NC-Slide. **Solution 12** contains a dye, DAPI staining all cell nuclei after lysis with **Solution 10**. After loading the NC-Slide it is placed in the NucleoCounter® NC-3000™ where cell concentration is determined.
The nominal depth of the chambers in the NC-Slides is 100 µm, with 90 % of all chambers being in the range from 90-110 µm. If higher precision in cell count is needed we recommend using the application "Count of Aggregated Cells Assay" using the volume calibrated Via1-Cassette™.

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
- NC-Slide A2™ or NC-Slide A8™
- **Solution 10** (Lysis buffer)
- **Solution 12** (500 µg/ml DAPI)

1. Add 1 volume of **Solution 12** into 99 volumes of **Solution 10**. E.g., add 10 µl of **Solution 12** to 990 µl **Solution 10**. Note: this mixture is stable for at least one week at 4° C.
2. The cell suspension is mixed to obtain a homogenous suspension. Pipette a representative cell sample from the cell suspension into a microcentrifuge tube.
3. Add one volume of the mixture of **Solution 12** and **Solution 10** to the microcentrifuge tube with the cell sample. E.g., if the volume of the cell sample is 100 µl then add 100 µl of the mixture of **Solution 12** and **Solution 10**. Mix by pipetting.
4. Load ~30 µl or ~10 µl of each sample into the chambers of the NC-Slide A2™ or NC-Slide A8™, respectively. Place the loaded slide on the tray of the NucleoCounter® NC-3000™ and select "**Count of Aggregated Cells Assay**" and sample unit **NC-Slide A2** or **NC-Slide A8** and press RUN.

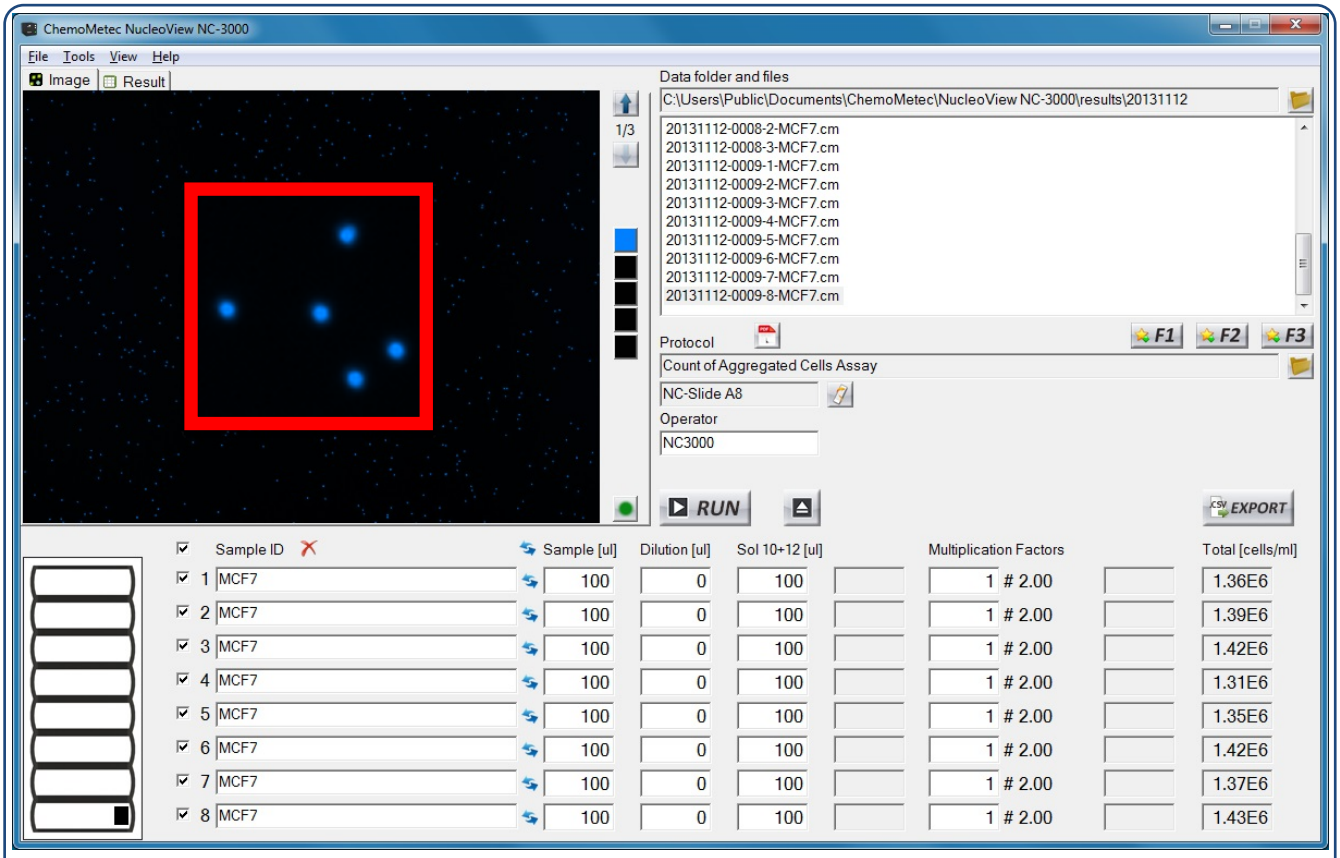
After analysis the concentrations (cells/ml) of all cells is displayed in the result tab. The displayed cell concentrations have been compensated for the two time dilution caused by **Solution 10** and **Solution 12**. If the cell sample has been further

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.

Notes

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.



Determination of cell concentration of aggregated MCF7 cells. The cells were disaggregated by adding **Solution 10** and analyzed using the Count of Aggregated Cells Assay. The total cell population is stained with DAPI and appears blue. An insert shows a close up of a part of the image.

Trouble shooting

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 Raw Data”. Inspect the gates displayed in the Plot Manager. If the gating is inappropriate right click on the image file in question again and choose “Start Protocol Adaptation Wizard”. Adapt the gate(s) to cover the cell population (do not include debris and very large objects) and save the changes to a new protocol. Note that the user is responsible for defining appropriate gating of the particular cell line.

Inappropriate loading of the NC-Slides:

Due to variations in chamber volumes the exact amount needed to fill the chamber may vary. Make sure that the chamber is completely filled and that no excess liquid spreads into other chambers or onto the top of the cover slip. Furthermore, avoid introduction of air bubbles into the chambers. Insufficient filling and air bubbles may cause cell movement compromising the quality of the image analysis.

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, NC-slide and the NucleoCounter® NC-3000™ instrument and might depend on the type of cells being analyzed. Refer to the NucleoCounter® NC-3000™ 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.

Copyright