Application note No. 0273

NucleoCounter® NC-250™

Viability and Cell Count – Method 2 using NC-Slides™

Product description

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

Application

Method 2 Assay is an alternative analysis to address changes triggered by prolonged cell incubation in bioreactors. The NC-Slides™ and **Solution 18** used together with the NucleoCounter® NC-250™ facilitates determination of viability and concentration of cell suspensions by measuring cell counts (total and non-viable) per volume. The nominal depth of the chambers in a NC-Slide™ is 100 µm, with 90 % of all chambers being in the range from 90-110 µm.

Introduction

Cells cultivated in bioreactors for long periods (e.g. <240h) without media exchange will tend to show unusual fluorescence patterns of Acridine Orange (AO) and DAPI when compared to fresh samples. This includes formation of small cell-like objects positive for AO but negative for DAPI. The opposite can also be true, with events that are positive for DAPI or Hoechst but negative for AO.

Method 2 accounts for presence of small cell-like objects by allowing inclusion of dim fluorescence intensities that would otherwise be excluded as debris. Also, it permits inclusion of cells stained only with DAPI but not with AO. Note that Method 2 is a more permissive analysis and should only be used in cases where small cell-like objects positive for AO or DAPI are expected.

Procedures

Materials needed

- Cell sample to be counted
- NC-Slide A2™ or NC-Slide A8™
- **Solution 18**

1. Engage NucleoView software to select the **"Viability and Cell Count – Method 2 Assay"** and sample unit **NC-Slide A2™** or **NC-Slide A8™**. Select chambers to analyse and insert Sample ID, if applicable.
2. Mix the cell suspension by inversion or flicking in order to obtain a homogenous suspension and pipette a representative cell sample (recommended >50 µl) from the cell suspension into a microcentrifuge tube.
3. Add one volume of **Solution 18** into 20 volumes of the cell suspension. As example, if the volume of the cell suspension is 100 µL then add 5 µL **Solution 18**. 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-250™ and press RUN.

After analysis the viability (in percent) and the concentrations (cells/mL) of all cells and non-viable cells is displayed. The analysis compensates for the dilution caused by the addition of **Solution 18**. If the cell sample has been further diluted or

concentrated and the user has entered the volumes into the user interface, the returned cell concentration will be for the original cell concentration.

Notes

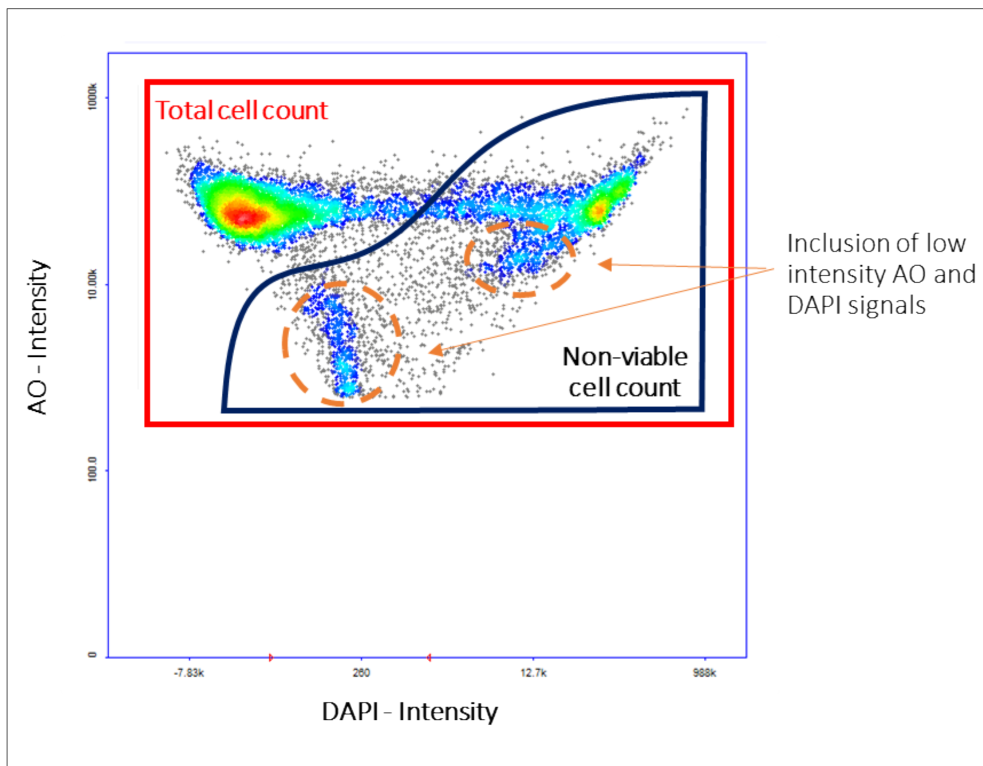
To assure reliable results, the cell concentration of the counted cell suspension should be in the range of $5{\cdot}10^4$ cells/ml to $5{\cdot}10^6$ cells/ml. If the cell concentration is above $5{\cdot}10^6$ cells/ml, the cell suspension can be diluted with growth media or PBS to achieve the desired concentration. If the concentration of cells is below $5{\cdot}10^4$ cells/ml, it may be increased by centrifugation followed by resuspension of the pellet using growth media or PBS.
Method 2 does not distinguish between small cell-like objects and enucleate AO-positive cellular debris. If cellular debris is present in the sample and the aim is to include only nucleated events, we recommend switching to the **“Aggregated Cell Assay”**. In such case, note that nuclei-based cell count in samples containing vesicular debris might differ from results generated using a light microscopy-based method, which tend to count enucleate events as cells.

Viability

The viability is calculated by the NucleoCounter software as follows:

$$\%\ \text{viability} = \frac{\text{Ct-Cnv}}{\text{Ct}} \cdot 100\%$$

% viability	The percentage of viable cells in the sample
$\mathbf{C_t}$	The total concentration of cells
$\mathbf{C_{nv}}$	The concentration of non-viable cells



Method 2 analysis includes low intensity Acridine Orange (AO) and DAPI. Cells were harvested, loaded into a chamber in NC-slides™ and analyzed using the “Viability and Cell Count – Method 2 Assay”. Total cell count includes all identified objects, regardless of intensity differences (left). Dead cell count includes low intensity AO together with low and high intensity DAPI.

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 Data 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.

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-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, NC-slide and the NucleoCounter® NC-250™ instrument and might 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
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
Copyright © ChemoMetec A/S 2003. All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior written consent of ChemoMetec A/S, Gydevang 43, DK-3450 Allerod, Denmark.