

NucleoCounter® NC-3000™ system Application note No. 001. Rev. 1.1

Quantitative analysis of GFP transfection efficiency

Multiplex assay for simultaneous measurement of transfection efficiency, cell concentration and viability

Product description

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

Application

The NucleoCounter® NC-3000™ system with PI-Cassettes enables easy quantification of green fluorescent protein (GFP) transfection efficiency along with determination of total cell concentration and viability. The system can be used for a wide range of mammalian cells.

The software offers a variety of features such as calculation of transfection ratio, determination of cell

viability and cell concentration and documentation of the results.

Introduction

In order to determine the concentration of transfected cells, total concentration of cells and transfection ratio, a suspension of cells transfected with GFP is stained with [Solution 1](#). Approximately 50 µl of the stained cell suspension is then drawn into the PI-Cassette. The PI-Cassette is placed in the NucleoCounter® NC-3000™ where the concentration of transfected cells and the total concentration of viable and nonviable cells is determined and the transfection efficiency and viability are calculated.

Procedures

The NC-3000 assay for determining GFP transfection efficiency includes viability determination. The transfection efficiency and cell viability are automatically calculated by the NC-3000™ software. However, the formulas used for calculating the transfection efficiency and viability are shown here.

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). Although NC-3000™ is able to count aggregated cells, the accuracy is higher for single cell suspensions.

Materials needed

- GFP transfected cells
 - [Solution 1](#)
 - PI-Cassette™
1. Pipette a representative cell sample from the cell suspension into a microfuge tube. Add one volume of [Solution 1](#) into 20 volumes of the cell suspension. E.g., if the volume of the cell suspension is 190 µL then add 10 µL [Solution 1](#). Mix by pipetting.
 2. Load the PI-Cassette with the mixture of cells and VitaBright-48 by immersing the tip of the cassette into the stained cell suspension and pressing the piston.
 3. Immediately place the loaded PI-Cassette on the tray of the NucleoCounter® NC-3000™ and select the **GFP transfection efficiency protocol**.

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After approximately 30 seconds the transfection efficiency (in percent), viability (in percent) and the total cell concentration (cells/mL) are shown in the result box on the computer. A histogram showing the GFP intensity distribution is also displayed.

Note

To assure reliable determination of transfection efficiency using the NucleoCounter® NC-3000™ system, it is recommended that the total cell concentration of the cell suspension should be in the range of $5 \cdot 10^4$ cells/mL to $1 \cdot 10^7$ cells/mL. Moreover, the concentration of transfected cells should preferably also be within this range.

If the concentration of cells (total cells and/or transfected 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 or PBS media. The resuspended cell sample is then treated as described above.

If the total cell concentration is above $1 \cdot 10^7$ 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 in the procedure.

Transfection efficiency

Calculation of transfection efficiency is performed by the NC-3000™ software as follows.

$$\% \text{ transfection efficiency} = \frac{C_{FP}}{C_v} \cdot 100\%$$

% transfection efficiency The percentage of cells expressing fluorescent protein in the cell suspension.

C_{FP} The concentration of cells expressing fluorescent protein in the PI-Cassette.

C_v The total concentration of viable cells in the PI-Cassette.

Viability

The viability is calculated by the NucleoCounter software as follows:

$$\% \text{ viability} = \frac{C_t - C_{nv}}{C_t} \cdot 100\%$$

% viability The percentage of viable cells in the cell suspension.

C_t The total concentration of cells in the PI-Cassette.

C_{nv} The concentration of non-viable cells in the PI-Cassette.

Handling and storage

For handling and storage of ChemoMetec instruments and reagents 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 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

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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 analysed. 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.

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