Application note No. 3023

NucleoCounter® NC-3000™

GFP transfection efficiency assay using Hoechst 33342 and propidium iodide

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 enables easy quantification of enhanced green fluorescent protein (EGFP) transfection efficiency. The system is optimized for relatively high expression levels (e.g. using the hCMV promoter) and can be used for a wide range of cells. The software offers a variety of features such as calculation of transfection ratio, presentation of GFP fluorescence intensity, and documentation of the results.

Introduction

In order to determine the transfection ratio, a suspension of cells transfected with GFP is stained with propidium iodide (PI) and Hoechst 33342. PI stains nonviable cells and Hoechst 33342 stains all cells. After staining cells are loaded into either of two types of ChemoMetec slides: the 2-chamber NC-Slide A2™ or the 8-chamber NC-Slide A8™. Samples are analyzed using the NucleoCounter® NC-3000™ system where the transfection ratios of viable, nonviable and all cells are 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). Although NC-3000™ is able to analyse aggregated cells, the accuracy is higher for single cell suspensions.

Materials needed

- GFP transfected cells[1]
- **Solution 15** (Hoechst 33342, 500 µg/ml)
- **Solution 16** (Propidium Iodide, 500 µg/ml)
- **NC-Slide A2™** or **NC-Slide A8™**

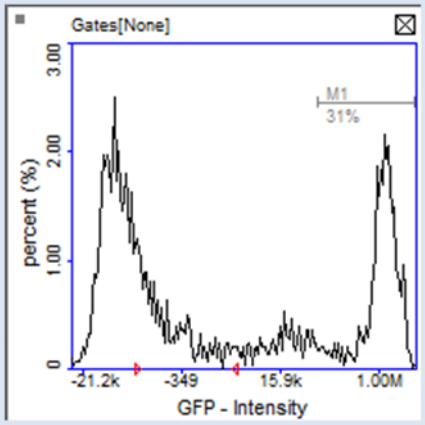
[1] Cells provided by the user.

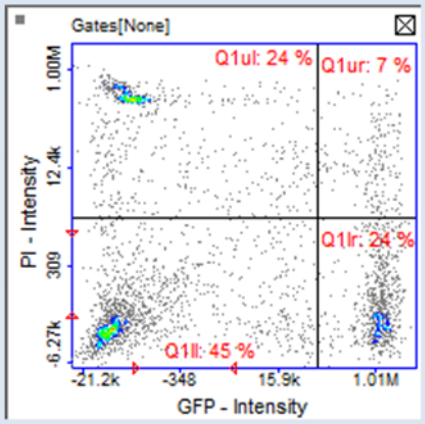
1. Pipette 96 µl representative cell sample from the cell suspension into a microcentrifuge tube. Add 2 µl **Solution 15**. Mix by pipetting.
2. Incubate cells at 37 °C for 15 minutes using a heating block. This step is crucial, thus comply with the specified incubation time and temperature!
3. After incubation add 2 µl **Solution 16**. Mix by pipetting.
4. Depending on the number of samples a 2-chamber slide (**NC-Slide A2™**) or an 8-chamber slide (**NC-Slide A8™**) can be used.
   a. **NC-Slide A2™**: Load approximately ~30 µl of each of the cell suspensions into the chambers of the slide. Place the loaded slide on the tray of the NucleoCounter® NC-3000™ and select "**GFP Transfection Assay – Hoechst and PI**" and sample unit **NC-Slide A2** and press RUN.

b. **NC-Slide A8™**: Load approximately ~10 µl of each of the cell suspensions into the chambers of the slide. Place the loaded slide on the tray of the NucleoCounter® NC-3000™ and select "**GFP Transfection Assay – Hoechst and PI**" and sample unit **NC-Slide A8** and press RUN.

After image acquisition and analysis scatter plots and histograms showing information about e.g. GFP fluorescence intensity will be displayed on the PC screen, and in the result tab will contain the results consisting of transfection ratios (as percentage) for viable, nonviable and all cells. Moreover, as a summary of the histogram the mean, standard deviation and main number (the most frequently occurring value) of GFP fluorescence are also shown in the result tab.

Note
To assure reliable determination of transfection efficiency using the NucleoCounter® NC-3000™ system, it is recommended that the total cell concentration in the sample should be in the range of $5 \cdot 10^4$ cells/ml to $5 \cdot 10^6$ cells/ml.
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 above.
If the total 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 in the procedure.





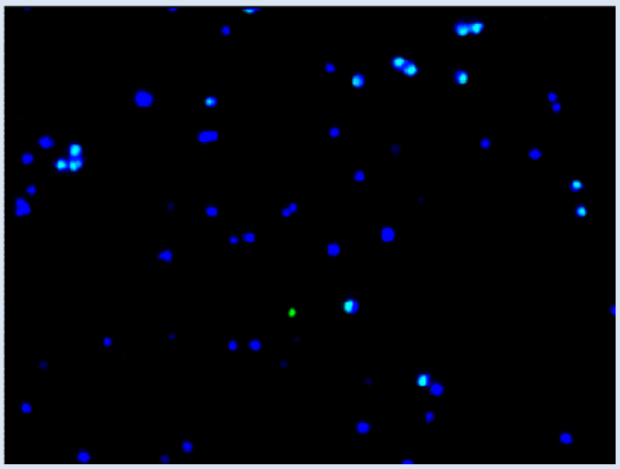


Image	GFP Transfection - Hoechst PI MCF-7
Chamber	1
Estimated viability	80 to 90 %
Transfection ratio - viable cells [%]	35
Transfection ratio - nonviable cells [%]	7
Transfection ratio - all cells [%]	31
GFP mean intensity	1.34E6
GFP standard deviation	8.67E5
GFP main number	3.24E4

24 hours after transfection with a GFP harbouring plasmid, cells were harvested, stained with **Solution 15** (Hoechst) and **Solution 16** (PI) and analyzed using the NC-3000™. A histogram (upper left) shows the GFP intensity of the cell population. A scatter plot (lower left) shows the GFP intensity versus the intensity of PI. The NC-3000™ aquired image is shown in the upper right corner. Green cells are GFP expressing cells, non-viable cells are red and blue cells are cells stained with Hoechst. The result tab will show information about transfection ratio, viability and GFP intensity.

Trouble shooting

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.

Inappropriate Hoechst-staining of cells:
The assay depends on successful staining with **Solution 15** (Hoechst-staining). In order to obtain this it is crucial to follow the protocol carefully. Thus, in step 2 it is mandatory to incubate at 37 °C for 15 minutes using a heating block. Do not use other Hoechst variants than Hoechst 33342/**Solution 15**.

Overexposed images due to very high GFP expression levels
The assay has been optimized to relatively high expression levels of GFP. However, we have noticed that cells which express very high levels of GFP may cause neighboring cells to appear falsely GFP positive. This will result in overestimation of transfection efficiency. This problem occurs mainly when having a combination of strongly GFP expressing cells AND high cell concentrations. Thus, if the GFP signal is extremely bright make sure that the cell concentration is below $1 \cdot 10^6$cells/ml.

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 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.

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