

Performance of NucleoCounter® NC-250™

The NucleoCounter® NC-250™ offer precise measurement of cell concentrations and viability

Introduction

The most commonly used method to determine cell concentration today, although highly imprecise, is still manual counting of cells in hemocytometers. Advancements in microscopy and software image analysis have made it possible to develop automated image based cell counting, providing easy, objective and accurate results.

The NucleoCounter® NC-250™ and its performance are described in this technical note in terms of accuracy, precision and measurement range.

The NucleoCounter® NC-250™ system

If high quality in cell counting is important for you, the NucleoCounter® NC-250™ is the right instrument for you. The NucleoCounter® NC-250™

provides precise and objective cell counts, in a fast and easy manner. To perform a viability and cell count assay, you only have to add Solution 18 to the cell sample and load a slide, insert the slide into the NucleoCounter® NC-250™ and press 'Run' (Figure 1). After the analysis is completed the image and results are displayed showing the total cell concentration (in cells/ml) and the viability (in %). Furthermore, extended results are available giving the cell concentrations of viable and non-viable cells, the average cell diameter and the standard deviation on the determination of the cell diameter, together with information about the degree of cell aggregation ("clumpiness").

A



B

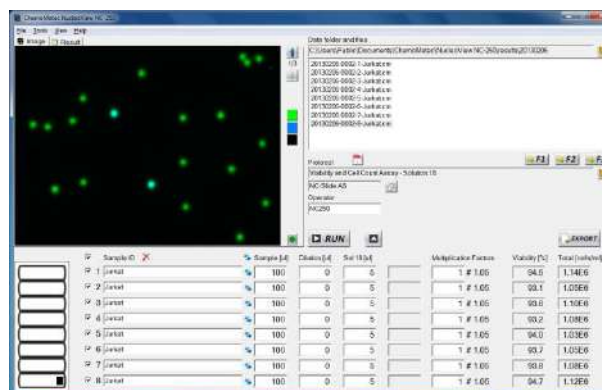


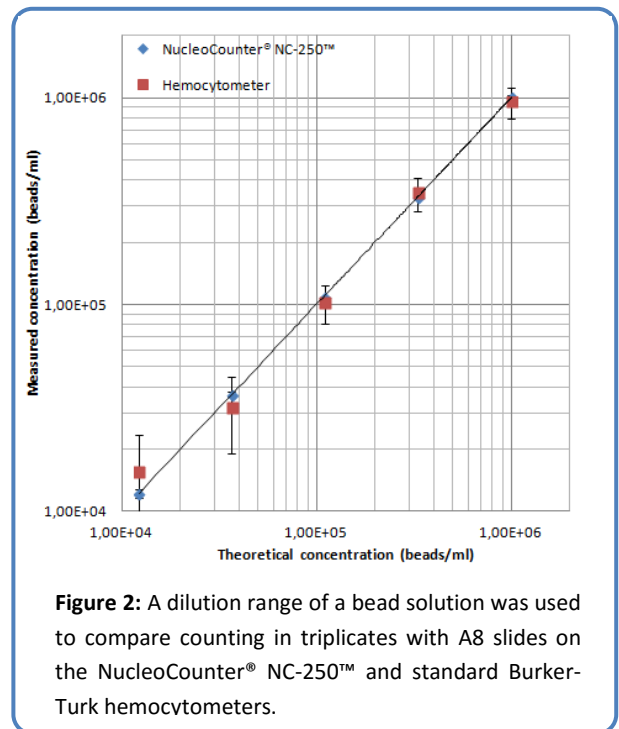
Figure 1: Viability and cell count assay with the NucleoCounter® NC-250™: (A) Mix cell sample with solution 18 and load the slide. Insert the slide in the instrument. (B) Press run and results are displayed continually for each chamber.

Key benefits of the NucleoCounter® NC-250™

- **World's lowest running costs:** Performing viability and cell counts in A8 chamber slides provides the world's lowest running costs on an automated viability and cell count analysis.
- **Objective cell count:** The automation of cell counting eliminates the individual subjective judgment of users, providing a standardized method that enables comparison of data between different laboratories.
- **Calibration free:** The instrument is calibrated by ChemoMetec and no subsequent calibration is needed.
- **Service free:** The construction of the NucleoCounter® NC-250™ contains no internal flow system to become contaminated or blocked.
- **Flexible software package:** Protocols can be adapted by the user to fit specific needs. The software is 21 CFR part 11 ready for regulated environments.

Performance

The NucleoCounter® NC-250™ produces very precise results with small average coefficients of variation (CV), indicating a high level of reproducibility when counting cells. The average percent CV, calculated by measuring cell concentrations of approximately 1.1×10^6 cells/ml in A8 chamber slides of 8 different cell lines (n=3), is



2.05 % (standard deviation ± 0.43). The precision of the determination of the cell count is dependent on the number of cells counted. It is normally assumed that counting random events follows the Poisson distribution, according to which the expected standard deviation is equivalent to the square root of the number of counted events. Therefore, the theoretical CV of the NucleoCounter® NC-250™ instrument using A8 slides at the cell concentration of 1.1×10^6 cells/ml can be calculated to be 1.96 %.

Table 1: List of cell types verified on the NucleoCounter® system.

Cell type	Species	Tissue	Remarks
BSC-1	African green monkey	Kidney	
CEF*	Chicken	Embryo	
CHO	Chinese hamster	Ovary	
COS-7	African green monkey	Kidney	
Embryonic stem cells*	Human	Embryo	
HEK-293	Human	Kidney	
HeLa	Human	Cervix	
Hematopoietic cells*	Mouse	Bone marrow	
HepG2	Human	Liver	
Jurkat	Human	Blood	
K562	Human	Bone marrow	
MCF7	Human	Breast	
NHBE*	Human	Bronchi	Assay requires lysis
NHDF*	Human	Skin	Assay requires lysis
NHEK*	Human	Skin	Assay requires lysis
NIH/3T3	Mouse	Embryo	
SF9	Fall army worm	Ovaries	
SVF cells	Human	Adipose tissue	
T-cells (purified)*	Human	Blood	
U2OS	Human	Bone	
Vero cells (grown on micro carriers)	African green monkey	Kidney	Assay requires lysis
WEHI-164	Mouse	Fibrosarcoma	
Whole blood*	Human	Blood	Assay require RBC lysis and incubation
YAC-1	Mouse	Blood	

* Primary cells.

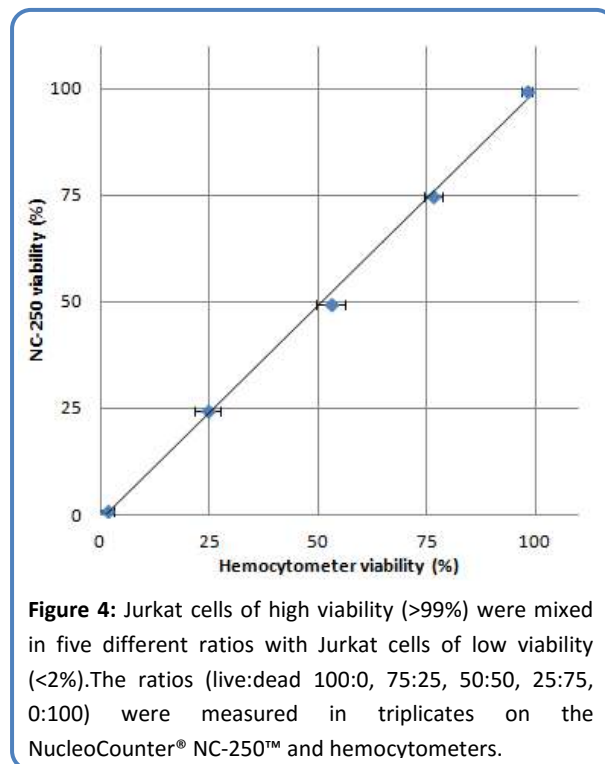
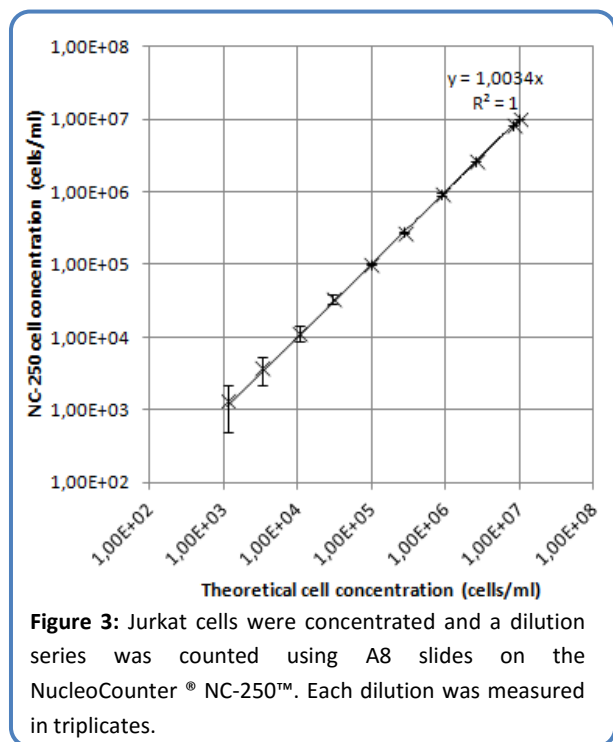
In the assays that require lysis, a solution is added to the sample for the total cell count. An additional chamber is required in this assay to measure the viability. In the whole blood assay, lysis of red blood cells (RBCs) is obtained by addition of a solution and an incubation step before analysis. This assay only requires one chamber for a viability and cell count analysis of leukocytes.

Cell concentration measurements performed by manual counting in hemocytometers commonly gives CVs above 10% when counts are performed by several individuals in blinded experiments. Comparing NucleoCounter® NC-250™ counts using

A8 slides on a dilution range of a bead solution with manual counts performed with Burker-Turk hemocytometers shows a very good correlation between the results obtained with the two counting methods (Figure 2).

Cell concentration range

The working range of the NucleoCounter® NC-250™ is 5×10^3 to 1×10^7 cells/ml in the tested solution, and the optimal range is 5×10^4 to 5×10^6 cells/ml. A warning will be given when analysis results are outside the optimal range. The working range is determined by the linear range where measurements can be performed (Figure 3). However, the majority of mammalian cell lines display non-linear counting tendencies above 5×10^6 cells/ml and, on the basis of this, the upper limit of the optimal range was set. The lower limit of the optimal range is set at 5×10^4 cells/ml, due to that the determination below this cell concentration becomes increasingly uncertain, again due to low number of cells counted as described above. The theoretical CV at the cell concentration of 5×10^4



cells/ml is 9.1% when using A8 slides. It is recommended that the linear range is determined for the employed cell lines to ensure reliable results. Counting of a variety of different cell types has been verified in the NucleoCounter® system (Table 1).

Viability range

The NucleoCounter® NC-250™ provides viability measurements in the full range from 0% to a 100%. DAPI is used to quantify the number of non-viable cells. There is good correlation between viabilities determined by the NucleoCounter® NC-250™ and the hemocytometers (Figure 4).

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, cassettes 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

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