

# Fast, efficient and precise cell counts with the NucleoCounter™

**With the NucleoCounter™ automatic cell counting system obstacles like viability determination, reliability, instrument calibration and hemocytometer cleaning are all resolved.**

**The NucleoCounter™ is maintenance and calibration free, with extreme ease of use.**



## Introducing the NucleoCounter™

### Introduction

ChemoMetec A/S has developed and patented a new technology which addresses the problems of conventional cell counting. The NucleoCounter™ is currently optimized to count cultured mammalian cells in research and production applications (T-flasks, bioreactors, micro carriers etc.). The integrated fluorescence microscope in the NucleoCounter™ is designed to detect signals from the fluorescent dye, propidium iodide (PI), bound to cell nuclei. This result of the NucleoCounter™ represents either a total or non-viable cell concentration, depending on the preparation of the cell sample.

## Simple as 1-2-3

### System description

The system consists of the NucleoCounter™, the NucleoCassette™ containing immobilized propidium iodide (PI) and the reagents A and B (lysis/dis-aggregation buffers). In addition the operator can use the NucleoView™ PC software application for documentation, image viewing and data processing.



## Handling the NucleoCounter™

Compared to other cell counting systems the NucleoCounter™ is fast, efficient and reliable. The determination of total cell count involves sample preparation and sample analysis. During sample preparation, cell nuclei are released from the cells by disrupting the plasma membrane by the addition of cell lysing reagent. The cell lysate is subsequently loaded into a NucleoCassette™ where the nuclei are stained with PI. The NucleoCassette™ is then placed into the NucleoCounter™ for analysis. During analysis the fluorescent signal is registered and correlated to a total cell count. The total cell concentration in the NucleoCassette™ chamber is presented in the NucleoCounter™ display as cells per ml. To obtain the total cell concentration prior to dilution (with reagents A and B) the result must be adjusted with the multiplication factor. The NucleoView™ software can automatically present the dilution-adjusted result.



### Sample preparation - Time: 30 seconds

1. Pipette a representative cell sample from the cell suspension (e.g. 200  $\mu$ l). Add equal volume reagent A (e.g. 200  $\mu$ l) and vortex. Then add the equal volume (e.g. 200  $\mu$ l) reagent B and vortex.
2. Load the NucleoCassette™ with approximately 50  $\mu$ l cell lysate by immersing the tip of the cassette into the cell lysate and pressing on the piston.

### Sample analysis - Time: 30 seconds

3. Place the NucleoCassette™ in the NucleoCounter™ and press the "Run" button. After less than 30 seconds the estimated total cell concentration appears in the NucleoCounter™ display. The data are optionally presented on a PC by using the NucleoView™ software application.

## Limitations of present methods of cell counting

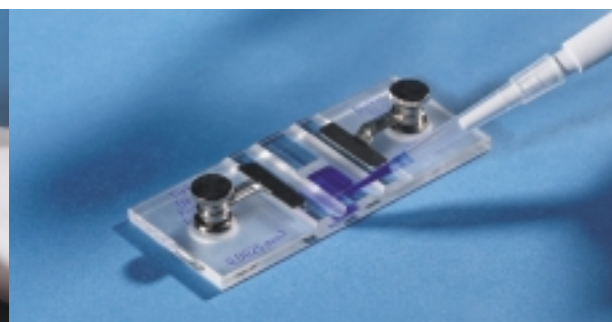
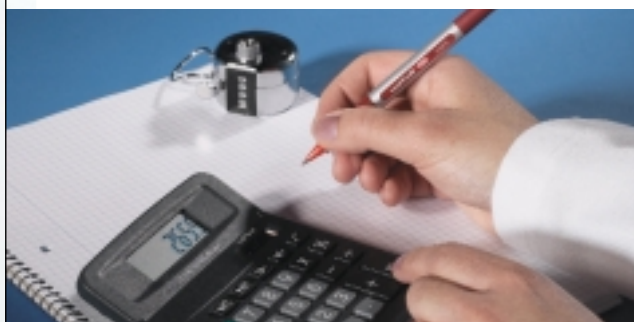
### Hemocytometer: manual cell counting methods

Several different dyes are explored in manual cell counting using the hemocytometer. The trypan blue exclusion method is widely used in laboratories for viability determination. Trypan blue only enters cells with impaired plasma membranes. The membrane of viable cells excludes the dye, and stained and non-stained cells are visualized in a light microscope. A drawback with this method is, however, that trypan blue has staining artifacts, and furthermore that the proportion of stained cells increases with incubation time (Jones, K.H., Senft, J.A. J. Histochem. Cytochem. 33, 77-79 (1985)). Cells are classified as non-viable or viable due to the subjective judgment of the operator. Fluorometric cell viability determination using dyes like acridine orange and propidium iodide are generally regarded as superior to the trypan blue exclusion method with regard to stain specificity and incubation stability (Mascotti, et al. Transfusion 40, 693-697 (2000)). However, the cost of fluorescence

microscopes has discouraged most laboratories from using this method. The naked eye can only differentiate between cells in a limited concentration range in the hemocytometer chamber. This, combined with the potential problem of cell aggregation and the small volume analyzed in a hemocytometer, means that most laboratories find it difficult to obtain high precision using the manual methods.

### Automatic cell counting methods

Precision has been slightly improved in several automatic methods of cell counting, but until now these methods have not solved the critical points to satisfaction. To date, automatic viability determinations have been based either on the trypan blue exclusion method or on differentiation between cell sizes using impedance. Both methods compromise the final result. Many of these systems need frequent calibration for cell size or morphology prior to use or are generally regarded as costly concepts.



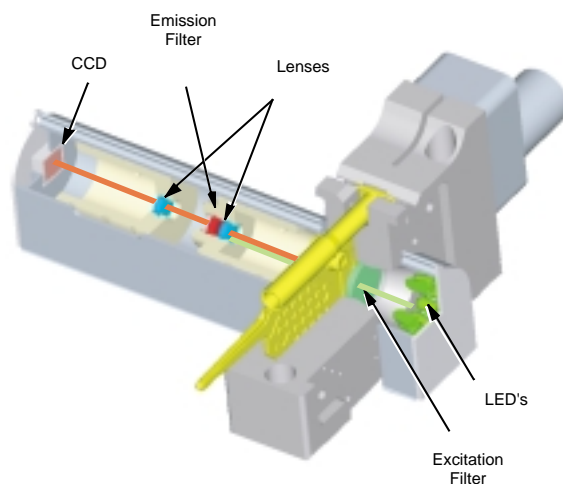
## The NucleoCounter™ cell counting system

The core of the system is a novel integrated fluorescence microscope, comprising an excitation light source of light emitting diodes (LED's), optics (including lenses, excitation and emission filters), and a charged coupled device (CCD) camera. The fluorescent microscope is optimized to excite PI with intense green light and subsequently detect the red light emitted from the excited molecules with a CCD camera.

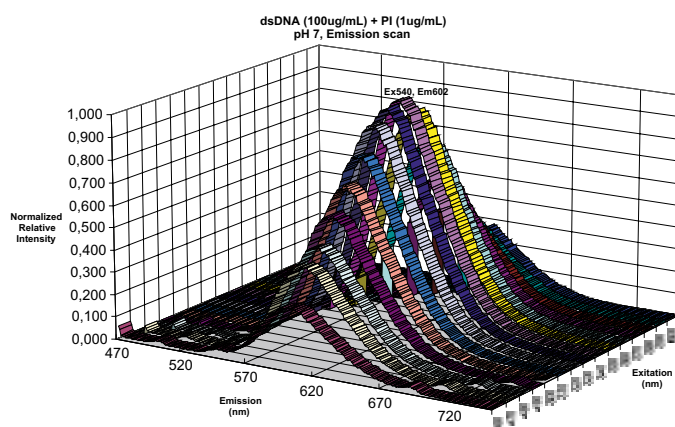
Detected signals are correlated to a cell count, which is presented to the user in the built-in display. Optionally, the image and result can be transferred to a PC for viewing and documentation purposes using the NucleoView™ software application.

## The integrated fluorescence microscope in the NucleoCounter™

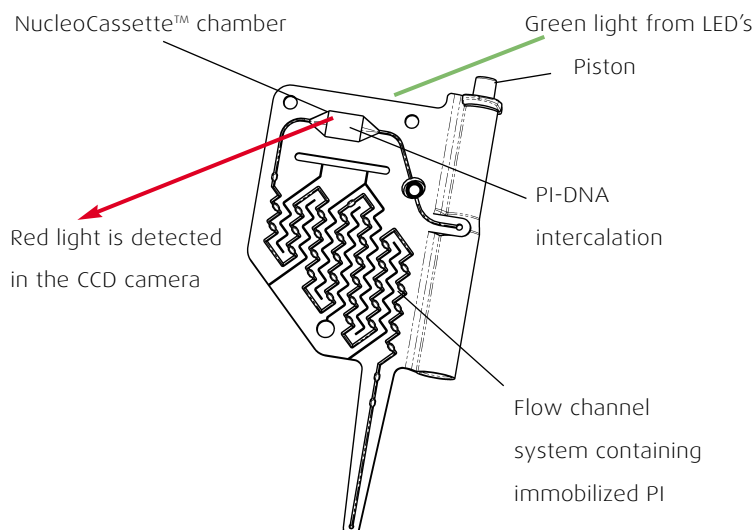
The interior of the NucleoCounter™ is basically a fluorescence microscope. The NucleoCassette™ is placed into the fixture. LED's emit green light in order to excite the PI-DNA intercalation. The excitation filter sorts the green light and the emission filter sorts the PI-DNA emitted red light. The CCD camera placed to the left in the NucleoCounter™ interior registers the red light and the signals are correlated to the cell count. The magnification is approximately 1.



The excitation maximum of PI moves approximately 30-40 nm into the green area after intercalation with dsDNA. The emission maximum moves only 15 nm (red light), but the fluorescence signal is intensified 20 – 30 times the emission from the PI alone.



The accessible cell nuclei are automatically stained within the NucleoCassette™. After placement in the fixture of the NucleoCounter™, the stained mixture is transferred to the NucleoCassette™ chamber. Green light excites the PI-DNA intercalation and the red light emitted is registered in the CCD camera for correlation into a cell count.



## Using the NucleoCassette™

PI is immobilized in the interior of the disposable NucleoCassette™. To load the cassette its tip is immersed into the cell lysate, the piston is pressed and the lysate is aspirated into the cassette. The lysate dissolves the PI and cellular DNA is stained. After placement in the NucleoCounter™ the stained nuclei are automatically transferred to the NucleoCassette™ chamber.

After use the NucleoCassette™ can be safely disposed of as biological waste with the PI safely enclosed. Unlike flow systems and hemocytometers the system requires no cleaning.

## Propidium iodide advantages

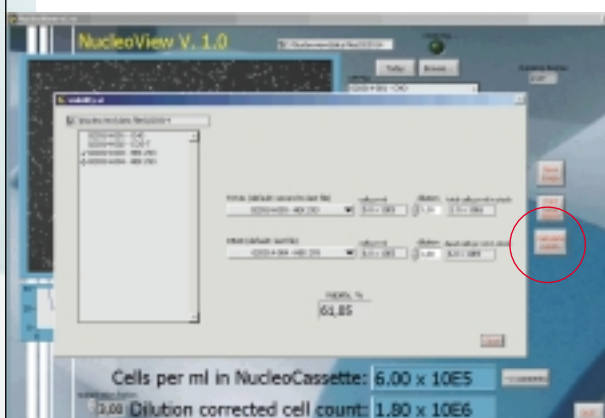
### **Propidium iodide, a nucleic acid intercalating fluorescence dye.**

Several laboratories deploying manual cell counting methods and a fluorescence microscope use the well-characterized PI to stain cell nuclei. Excitation of PI occurs around 540 nm (green light) and PI fluoresces brightly around 600 nm (red light). The signal intensity is enhanced by a factor of 20-30 when PI is bound to DNA compared to unbound PI, which improves the signal to noise ratio. This is particularly useful since the solution contains free PI and cellular debris. The NucleoCounter™ detects PI-stained nuclei rather than cells. Since nuclei are virtually uniform in size regardless of cell type no calibration for varying cell size or morphology is required.

### **Using the NucleoCounter™ for viability determination**

A cell suspension consists of viable and non-viable cells as well as cellular debris. A viability determination (the percentage of viable cells per total cells in a cell suspension) is therefore often desired. By deliberately disrupting the plasma membranes of all cells in a sample by the pretreatment using reagents A and B, all nuclei are accessible to PI staining, independent of whether cells initially were viable or non-viable prior to cell lysis. Analyzing the cell lysates therefore results in an estimate of the total cell concentration in the NucleoCassette™ chamber. However loading a cell sample, without pretreatment, directly into the NucleoCassette™, only cells with pre-impaired plasma membranes are nuclei-stained. Analysis in the NucleoCounter™ then gives an estimate of the concentration of non-viable cells in the original cell suspension.

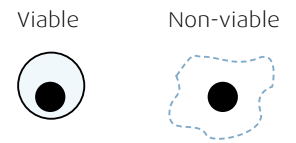
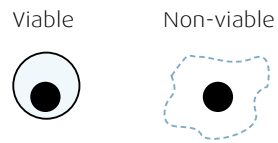
Based on these two measurements the viability can now be calculated by hand or by the push of a button, using the NucleoView™ software application.



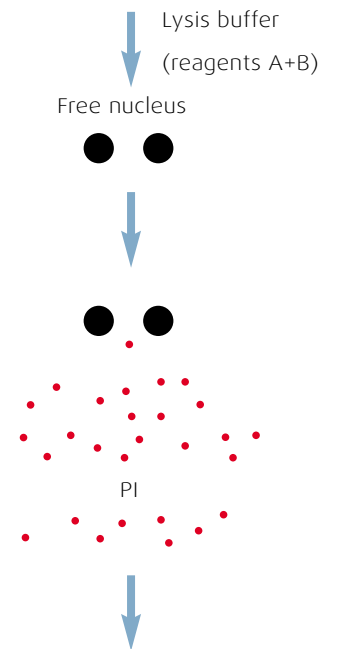
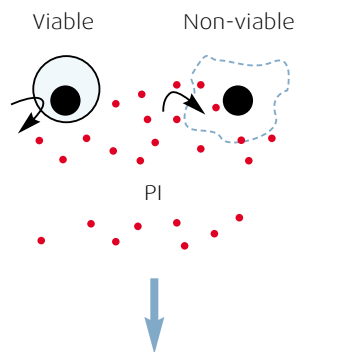
## Non-viable cell count

## Total cell count

### 1 Cell suspension



### 2 Cassette loading



### 3 Sample analysis



Plasma membrane

Intact

Disrupted

Disrupted

Disrupted

Cell count

**1**

**2**

Characteristics

Non-viable cell count

Total cell count

Propidium iodide is excluded from viable cells. This is utilized in the NucleoCounter™ to estimate the concentration of non-viable cells and the concentration of total cells in a suspension. From these two estimates the viability is easily calculated by hand or by using the NucleoView™ software.

$$V = \frac{C_t \times M_t - C_{nv} \times M_{nv}}{C_t \times M_t} \times 100$$

**V** : Viability

**C** : Cell concentration

**M** : Multiplication factor

**t** : Total cell count

**nv** : Non-viable cell count

## The NucleoCounter™ - precise and objective cell counting

### NucleoCassette™ chamber

Approximately 2 µl of sample is analyzed in one measurement, which is 10-20 times the volume, analyzed in a hemocytometer chamber. As such, this greatly improves the precision of the results compared to the hemocytometer method.

### Handling cell aggregates

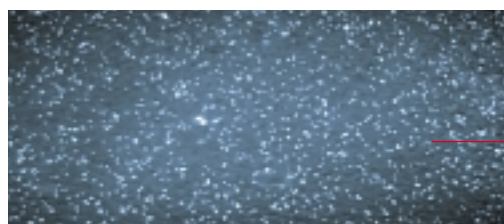
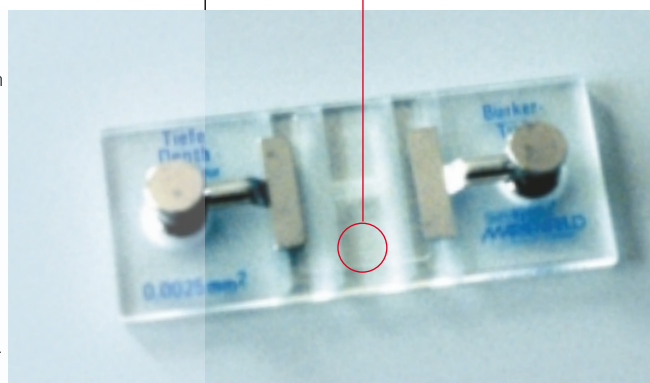
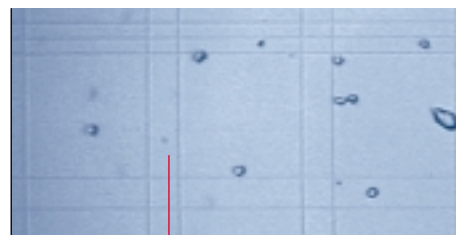
Whereas cell aggregation generally is a major problem, a cell count based on a single-nuclei measurement in lysed cell samples, rather than a cell suspension also greatly improves the statistical quality of the results. Using the NucleoCounter™, cell aggregates are dissolved by lysing cells with reagent A and B. The PI-DNA emission is identified in the software and signals representing single positioned nuclei are counted as cells. Signals representing an aggregate of nuclei are either corrected for, or this particular part of the image is rejected, depending on the degree of aggregation. This is also the case during a non-viable cell count. Although plasma membranes are not disrupted, cell aggregation is evaluated and only images of nuclei that can be differentiated are used.

The quality of the image is indicated by the “clumping degree” in the NucleoView™ software application.

### Objectivity

The counting of cells is operator independent in the NucleoCounter™. Most laboratories will agree that manual counting is a very subjective procedure. Each operator estimates the cell concentration differently. Consequently, the evaluation of non-viable or viable cells can give rise to disagreement among different operators. Counting cells with the NucleoCounter™ is an objective method and eliminates systematic differences between laboratories and operators, in particular in viability determination.

The automatic staining method utilized in the NucleoCassette™ resembles the trypan blue exclusion method. Propidium iodide also only enters cells with impaired plasma membranes. However, the NucleoCassette™ chamber comprises 2 µl, which is 10 to 20 times the volume contained in the hemocytometer chamber. These 2 µl are counted in one measurement due to the low magnification of the integrated fluorescence microscope.

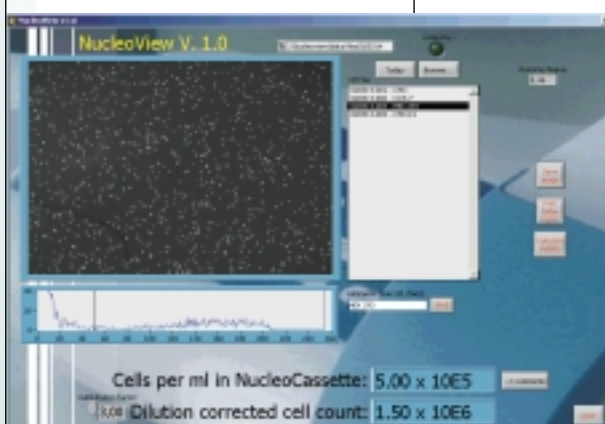


## How are data presented in the NucleoCounter™?

### The NucleoCounter™ display

Data are always presented in the NucleoCounter™ display as the number of cells per ml present in the NucleoCassette™ chamber.

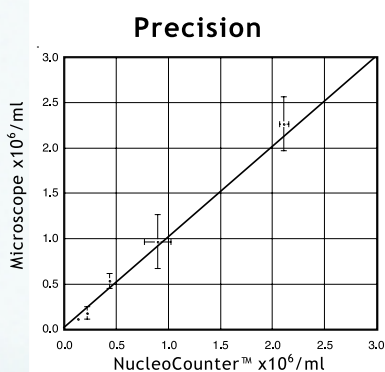
For documentation and further data processing the NucleoCounter™ system is supplied with a CD-ROM containing the NucleoView™ software application. By connecting the NucleoCounter™ to a PC and installing the software you will find data presentation that offers several advantages:



- 1 Printing and saving data for documentation purposes.
- 2 Captured visualized image for user evaluation. Cell or nuclei aggregates are easily observed in this image.
- 3 A number representing the clumping degree is presented for each sample analysis as a tool to evaluate the quality of the image, which is the basis for the cell count.
- 4 The cell concentration can be presented in three different number presentation formats, which are optional to the user.
- 5 The count is corrected for dilution by the multiplication factor in order to obtain the cell concentration in the cell suspension prior to dilution.
- 6 Viability calculation.

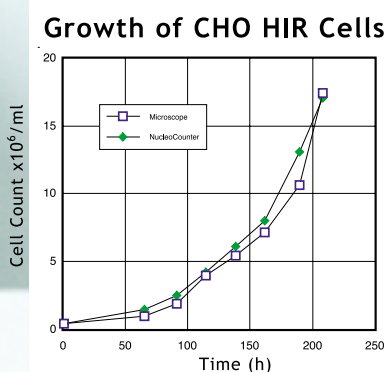
## NucleoCounter™ comparison with conventional cell counting methods

In collaboration with international pharmaceutical companies ChemoMetec A/S has compared the NucleoCounter™ to the routinely used manual light microscopy method (hemocytometer). The comparison showed that the NucleoCounter™ has a substantially better precision. The growth curve demonstrates that cell counts by the two methods are comparable.



### Precision

CHO HIR cell line samples with known cell concentration were counted manually in a light microscope using a hemocytometer, as well as automatically using the NucleoCounter™. Triple concentration determinations revealed that better precision is obtained with the NucleoCounter™ than with the manual method (generally the CV is less than 5% under normal conditions).



### Growth curve

The CHO HIR cells were cultured in T-flasks and eight flasks were inoculated simultaneously. At various time points cells were trypsinized and re-suspended in DMEM medium. Cells were lysed with 2% Triton X-100 and counted by the manual microscopy method, as well as in the NucleoCounter™. Growth curves were comparable.

## Key benefits

### ■ Objective cell count

Automation eliminates the subjective judgment of the operator. The NucleoCounter™ cell count is therefore an objective and operator-independent count. The standardized method provides the opportunity for directly comparing data between different laboratories.

### ■ Calibration free

Since cell counting using the NucleoCounter™ is based on stained nuclei, rather than on the whole cell, no calibration for cell size or morphology is required.

### ■ Maintenance free

The lid on the NucleoCounter™ excludes dust and there is no internal flow system to become contaminated or blocked.

### ■ Safe sample disposal

The NucleoCassette™ is disposable and can be disposed of safely as biological laboratory waste.

### ■ Great reliability and high precision

Great consideration has been given to reliability in the development of the NucleoCounter™ instrument. The solving of the traditional problems in cell counting methods has been addressed. Critical points such as precision have been greatly improved.

### ■ Viability determination

The NucleoCounter™ is based on fluorometric viability assays using the plasma membrane impermeable DNA binding dye, propidium iodide.

### ■ User-safety

The potentially hazardous propidium iodide is safely enclosed in the NucleoCassette™ thereby providing safety to the operator.

### ■ Specific and established method

By using the DNA binding fluorescent propidium iodide the cell count is based on a very specific signal. Propidium iodide is traditionally used in determination of the concentration of non-viable cells.

### ■ Small loading volume

Only 100 µl is needed to perform an analysis.

### ■ Large analysis volume

2 µl (10-20 times the volume in a hemocytometer) is analyzed in one measurement.

### ■ Documentation

The included NucleoView™ software application is ideal for data and image processing and for documentation purposes.

### ■ Compact instrument

The NucleoCounter™ fits into any laboratory facility due to its small size (38x26x22 cm).

### ■ Cost efficient

Analysis takes less than 30 seconds. The procedure is simple and easy to learn by laboratory staff. The NucleoCounter™ requires no daily cleaning or calibration and is maintenance free.



**Specificity**

**Loading volume**

**Analysis volume**

**Analysis time**

**Measurement range**

**Operation**

**Physical data**

**Power**

**Power consumption**

**Operation conditions**

**USB**

**Reagent**

**Storage**

**Stability**

**System requirements**

Technical specifications

**The NucleoCounter™**

The NucleoCounter™ counts mammalian cell nuclei stained with the DNA specific fluorescent dye, propidium iodide.

100 µl is recommended for NucleoCassette™ loading.

Approximately 50 µl is loaded into the cassette.

Approximately 2 µl of the sample loaded into the NucleoCassette™ are analyzed in the NucleoCassette™ chamber.

After pressing “Run” on the NucleoCounter™ the result will be displayed within 30 seconds.

The optimal range is 10<sup>5</sup> – 2 x 10<sup>6</sup> cells/ml in the NucleoCassette™ chamber.

Menu-controlled by means of keyboard and LCD display.

Weight 3 kg

Height 26 cm

Width 38 cm

Length 22 cm

Input 85 – 264 VAC, 48 – 63 Hz

Peak 25 W

Ready mode 10 W

Standby 2.5 W

Max. 80% relative humidity.

Temperature between 15 – 35°C.

USB, version 1.1.

Note: Does not support USB Hubs.

**The NucleoCassette™**

Each NucleoCassette™ contains approximately 2.8 µg propidium iodide.

Store the NucleoCassettes™ in a sealed bag at max. 30°C.

Stable for minimum 6 months.

**NucleoView™ software**

Windows 2000 operating system.

Windows 2000 compatible computer.

USB 1.1 port.

The information contained herein is to the best of our knowledge accurate and complete. However cell species and cell environments may vary in property. Therefore systematic and/or random deviation between estimates obtained by the NucleoCounter™ and other cell counting methods may occur. As such, nothing contained or stated herein, including results obtained from use of the NucleoCounter™ or NucleoCassette™, shall be construed to imply any warranty or guarantee. ChemoMetec A/S and affiliated companies shall not be held liable for damages, and customers shall indemnify ChemoMetec A/S and affiliated companies against liability flowing from use of potentially inaccurate data generated by the NucleoCounter™. It is recommended that all results obtained with the NucleoCounter™ be validated against appropriate reference methods and/or traditional laboratory methods at regular intervals.