

FAQ

NUCLEOCOUNTER® NC-100™

1. Why is the NucleoCounter calibration free?

- a. The NucleoCounter detects fluorescence signals from PI bound to the DNA of cell nuclei. Since the nuclei are uniform in size the variation in the detected PI fluorescence signal is within the factory set detection range, so that no calibration to cell type is necessary.
- b. The volume of sample measured in every single analysis is known and therefore no calibration to volume is necessary. The thickness of the measurement chamber of each NucleoCassette is measured during production and coded with black dots on each Cassette. The NucleoCounter reads the code. During production the NucleoCounter is calibrated for its viewing area, which will remain unchanged throughout the lifetime of the instrument. The product of the two factors (area multiplied by thickness) gives an accurate sample volume.
- c. The NucleoCounter optics, mechanics and electronics has negligible thermal and aging drift properties. The instrument therefore maintains its initial factory calibration. The light source and the linear actuator have been tested to last for more than 1 million analyses.

2. Why is the NucleoCounter maintenance and service free?

- a. The NucleoCounter is made of very durable and stable components. The light source and the linear actuator have been tested to last for more than 1 million analyses. As the NucleoCassette contains the entire flow system as well as the measurement chamber, neither maintenance nor service is required for the NucleoCounter.

3. Can results from different NucleoCounters be directly compared?

- a. Yes, results can be directly compared. All NucleoCounters instruments are calibrated to respond as the same Master NucleoCounter at ChemoMetec. At the same time the initial factory calibration is maintained throughout the lifetime of a NucleoCounter.

4. What is the precision of the NucleoCounter?

- a. The precision is described by the Poisson distribution, e.g. counting of random events, where the observed standard deviation is equal to the square root of the number of counted cells. The NucleoCounter will analyze about 2µl in each measurement. With the recommended sample preparation for total count (equal amounts of: Sample, Reagent A100 and Reagent B), 2 µl will contain 0.67 µl of the original Sample (33.3%). If the cell concentration of the sample prior to mixing with reagent is 2 x 10⁶ cells/ml, then the analyzed volume contains 1333 cells. The square root of 1333 is 37 and thus the relative precision, e.g. Coefficient of Variation (CV), of the result in this case is 2.7% expressed as one standard deviation.

5. What is the accuracy of the NucleoCounter?

- a. As a universal standard for cell counting does not exist the accuracy of the NucleoCounter is difficult to assess. On the other hand, one inherent property of the NucleoCounter is that all instruments respond in identical manner to detected signals. As a result of this no significant difference between two NucleoCounter instruments can be detected. So whatever the "accuracy" of the NucleoCounter is, the reproducibility of the NucleoCounter is only determined by the repeatability of the instruments. If a universal standard existed, then the accuracy in the example used above, is set by the Coefficient of Variation (CV) or in that case 2.7% expressed as one standard deviation.

6. After loading of the disposable cassette how long time can it then be left before measurement?

- a. Once the disposable cassette is loaded it is recommended to perform the analysis without delay.

7. Is the staining time with PI dependent on which types of cells are measured?

- a. No, since staining of the cell nuclei with PI occurs immediately and it is not dependent with different cell types.

8. What is the purpose of the black dots on the NucleoCassettes?

- a. The black dots on the NucleoCassettes indicate the thickness of the measuring chamber. During production of the Cassettes the thickness is measured and the Cassettes coded with black dots according to the thickness group. The code is read by the NucleoCounter during each measurement and is used to determine the volume analyzed.

9. What is the purpose of Reagent A-100 and Reagent B?

- a. The lysis Reagent A-100 is used to disrupt of the plasma membranes of the mammalian cells, rendering the nuclei susceptible to staining with propidium iodide (PI). Secondly, the reagent contributes effectively to disaggregation of cell clusters. The stabilizing Reagent B is used after Reagent A-100 to raise the pH value, allowing PI to stain the DNA of the cells more efficiently.

10. What is the purpose of Reagent C?

- a. Reagent C has the purpose of permeabilizing the cell wall and membranes of single-cell suspensions, rendering the nuclei susceptible to staining with propidium iodide (PI).

11. Why is the threshold of the signal intensity on the display of NucleoView fixed to 50?

- a. As all NucleoCounters are calibrated to respond like the Master NucleoCounter at ChemoMetec, a fixed threshold of the signal intensity is required.

12. Is the raw image or the processed image shown on the display of the NucleoView?

- a. The Raw image is shown on the display of the NucleoView program. This ensures that the presence of a contaminant in the image (e.g. dust particles and bubbles) is visual. A contaminant or bubble will not affect the counting of cells.

13. Why is there a difference in brightness of the signals on the display of NucleoView?

- a. The image of a cell will typically cover a rectangle of 1 by 2 or 2 by 2 pixels on the CCD. This will cause the intensities (brightness) of the cell signals to differ, however this variation is within the detection range of the algorithm in the NucleoCounter.

14. Why can the background intensity vary on the display of the NucleoView?

- a. The various samples analyzed can have different background level caused by fluorescence. The algorithm in the NucleoCounter detects the difference signal caused by PI (propidium iodide) bound to DNA from the cell, the variation in background levels will not affect the counting of the cells.

15. Once the NucleoCassette is loaded is there any risk of liquid spill from the tip?

- a. No. The piston inside the cassette acts as a pump (generating vacuum) when it is pressed during loading. This vacuum causes the sample to flow inside the cassette until the pressure at both ends of the liquid sample is the same. Therefore the piston acts as a "valve" when there is the same pressure on each side of the liquid sample. The gravity itself can not cause the liquid to move.

16. What is the detection principle used in NucleoCounter?

- a. The NucleoCounter Instrument Family all uses a dedicated fluorescence microscope. It detects propidium iodide (PI) bound to the DNA of cell nuclei.

17. When does the Actuator break down?

- a. Never, the actuator has been tested for more than 1 million analyses without breakdown or malfunction and during the complete test the velocity was kept at $\pm 1\%$ from the specified start value.

18. When do I have to put in a new light source?

- a. Never, the light source is made of highly durable LEDs (Light Emitting Diode). An LED is e.g. used as indicator lamps on most electronic equipment today, often as a red, green or yellow lamp. LEDs have very long lifetime. The LEDs in the NucleoCounter Instruments are tested at excessive conditions to have a lifetime of more than 1 million analyses.

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