Tech Note No. 0209. Rev. 2.0

NucleoCounter® NC-200™

Performance Qualification Protocol for the NucleoCounter® NC-200™ system

Product description
The NucleoCounter® NC-200™ system enables the user to perform automated cell counting and viability analyses of a broad range of eukaryotic cells.

Application
This protocol for the NucleoCounter® NC-200™ system is used for Performance Qualification (PQ).

Introduction
The NucleoCounter® NC-200™ is a fluorescence-based precision instrument for cell counting and viability analysis. This PQ procedure is designed to quantify the level of precision obtainable by the NucleoCounter® NC-200™ and operator combined. The PQ procedure employs fluorescent beads provided with the **NC-200 PQ Kit**.

This kit should **NOT** be considered a counting standard and can only be used to measure counting precision.

Principle
Counting precision is evaluated using three solutions of beads supplied with the **NC-200 PQ Kit**. Each of the three solutions contains a mixture of two bead types: The first type, constituting approximately 95%, will emit green light in the NC-200™ instrument, mimicking Acridine Orange (AO) positive live cells. The second bead type, constituting 5% of the beads, emit both blue and green light, mimicking the dual staining of dead cells. This combination of beads allows simultaneous evaluation of cell counting and viability precision at three different concentrations.

Each bead solution is counted in replicates of five to calculate the Coefficient of Variation (CV) as a measure of counting precision. The **NC-200 PQ Kit** includes a Package Insert (# 992-0039), a PQ checklist (# 994-0210) and a lot-specific Certificate of Analysis (#992-0040) that defines maximum accepted counting variation and CV limit.
Counting precision is deemed acceptable if the CV and average values are within the specified ranges.

A *Zero Count* is performed to ensure that there is no dust or other foreign particles on the lenses or camera, giving an artificially high cell count.

ChemoMetec A/S · Gydevang 43 · 3450 Allerod · Denmark · support@chemometec.com · www.chemometec.com ·

Materials

- **NC-200 PQ Kit** (P/N: 912-0014)
- 15 x Via1-Cassettes™ (P/N: 941-0012, 100 pcs.)

Protocol

- From the Protocol menu, select **Organism:** *Verification of NC-200* and **Protocol:** *Zero Count*.
  Note in Tech note 994-0210 if Zero Count was successful.
- From the protocol menu, select **Organism:** *Mammalian* and **Protocol:** *Viability and Cell Count Assay*.
- **Resuspend the beads by vigorous shaking**. This is to obtain a single-bead suspension, however; it is not possible to avoid a certain degree of bead aggregation. Vortex mixing CANNOT replace shaking! (For a more comprehensive description refer to tech note 994-0210).
- Load a Via1-Cassette™ with the bead suspension and carry out an analysis on the NucleoCounter® NC-200™ and note the results in the table provided in tech note 994-0210.
  Important: *Close the bottle and invert it gently before each of the five required measurements in order to avoid settling of the beads. Incorrect handling of the beads may lead to higher variation of the individual sample and the subsequent samples.*
- Calculate the average and the CV % of the obtained results to validate precision of the NucleoCounter® NC-200™.

It is not recommended to use one **NC-200 PQ Kit** for more than one PQ test. The volume of the bead suspension in each vial is deliberately kept greater than the volume required for five measurements, to mitigate the sample modification effect caused by the sampling. Deviating from the this may result PQ test fail.
Even with stringent procedures for apportioning the bead slurry into the vials, variation in the total bead concentration throughout a production lot is probable. If the total bead count average falls outside the acceptance range, while the CV is below the specified limit, the vial probably represents such variation and ChemoMetec® customer support should be contacted.

Statistical Analysis

To calculate whether the counting precision is in accordance with the specifications set by ChemoMetec A/S, four statistical parameters are to be calculated namely, percent viability average, bead count average, bead count standard deviation (SD) and bead count CV %. To calculate these values, the use of third-party spreadsheet software is recommended, however the principles underlying these calculations are listed below.

Average % Viability *= Sum of % Viability / Numbers of counts performed*
Average Bead Count *= Sum of Bead Count Results / Number of counts performed*
Bead Count Standard Deviation (SD):

$$SD = \sqrt{\frac{\Sigma(x-y)^2}{(n-1)}}$$

Where X *is the actual bead count,* y *is the average bead count and* n *is the number of counting assays performed.*
CV % *= (SD / Average bead count)* $\times$ *100*

Handling and storage

For handling and storage of ChemoMetec® instruments, reagents and cassettes 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-200™ 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-200™ system is FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE. The results presented by the NucleoCounter® NC-200™ system depend on correct use of the reagents, cassettes and the NucleoCounter® NC-200™ instrument and might depend on the type of cells being analyzed. Refer to the NucleoCounter® NC-200™ 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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