Application note No. 0034. Rev. 1.0

NucleoCounter® NC-100™

Cell counts – whole blood

Product description

The NucleoCounter® NC-100™ system is comprised of the NucleoCounter® NC-100™, NucleoCassettes™ and **Reagent C**. The NucleoCounter® NC-100™ is developed as a stand-alone instrument. Optionally the NucleoCounter® NC-100™ can be connected to a computer using the NucleoView software, which offers a variety of features such as documentation of the results.

Application

The NucleoCounter® NC-100™ system enables the user to perform automated cell counting analyses of mammalian leukocytes in blood samples containing erythrocytes. Samples should be analyzed within one hour after blood collection.

Principle

The procedure is used to determine the concentration of white blood cells (leukocytes) in a blood sample containing anticoagulant (e.g. heparin, EDTA, citrate, oxalate). A representative sample is withdrawn and mixed with **Reagent C**. The objective of **Reagent C** is to permeate the plasma membrane of the white blood cell facilitating staining of the nuclei with propidium iodide, which is coated on the inside of the NucleoCassette™. Moreover **Reagent C** also lyses the red blood cells (erythrocytes) which release hemoglobin to the supernatant. As the hemoglobin may interfere with the following analysis, the supernatant containing hemoglobin is either diluted (method 1) or removed (method 2).

Approximately 60 µl of the sample is then drawn into the NucleoCassette™. The NucleoCassette™ is placed in the NucleoCounter® NC-100™ where the cells are counted.

Procedures

Two procedures for determining the concentration of white blood cells are described. We recommend trying both methods and evaluating against existing cell counting procedures. For both methods it is important to use freshly collected blood samples (storage time < 1 hour).

Materials needed

- Cells to be counted
- PBS (Phosphate Buffered Saline)
- NucleoCassette™
- **Reagent C**

Method 1 (Diluting hemoglobin)

1. First dilute **Reagent C**; 250 µl **Reagent C** is mixed with 750 µl distilled water or PBS
2. Mix 100 µl blood with 900 µl PBS in a microcentrifuge tube
3. Mix by pipetting.
4. Withdraw 100 µl of the diluted sample.
5. In a new microtube mix the 100 µl of the diluted sample with 900 µl diluted **Reagent C** from step 1.

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6. Draw a sample of the diluted blood sample by inserting the tip of the NucleoCassette™ into the sample and pressing the piston.
7. Insert the NucleoCassette™ into the NucleoCounter® NC-100™, close the lid and press RUN. After approximately 30 seconds the total concentration of nuclei in the diluted sample is displayed on the NucleoCounter® NC-100™ and on the computer if one is connected to the NucleoCounter® NC-100™. Note that it is the number of cells pr. ml in the diluted blood sample, which is displayed. Therefore, in order to calculate the number of nucleated cells pr. ml in the original suspension, the displayed number must be multiplied by the multiplication factor. The multiplication factor is 100 assuming the suspension of cells has not been diluted prior to the procedure described above.

Method 2 (Removing supernatant containing hemoglobin)

1. Mix 100 µl blood with 700 µl PBS in a microcentrifuge tube.
2. Mix by pipetting.
3. Add 200 µl of undiluted **Reagent C** (this lyses the red blood cells).
4. Mix thoroughly (use a vortex mixer).
5. Spin down the white blood cells (e.g. 1600 rpm, for 5 min.)
6. Withdraw 900 µl supernant, be careful not to disturb the pellet.
7. Add 900 µl PBS and resuspend cells.
8. Draw a sample of the resuspended white blood cells by inserting the tip of the NucleoCassette™ into the sample and pressing the piston.
9. Insert the NucleoCassette™ into the NucleoCounter® NC-100™, close the lid and press RUN. After approximately 30 seconds the total concentration of nuclei in the sample is displayed on the NucleoCounter® NC-100™ and on the computer if one is connected to the NucleoCounter® NC-100™. Note that it is the number of nucleated cells pr. ml in the diluted blood sample, which is displayed. Therefore, in order to calculate the number of mammalian cells pr. ml in the original suspension, the displayed number must be multiplied by the multiplication factor. The multiplication factor is 10 assuming the suspension of cells has not been diluted prior to the procedure described above.

Note

To assure reliable results, it is recommended that the total cell concentration of the diluted cell suspension should be in the range of $5{\cdot}10^3$ cells/ml to $2{\cdot}10^6$ cells/ml. If the concentration of cells is below $5{\cdot}10^3$ 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.

Troubleshooting

Unexpected low cell count:

It is crucial to analyze samples within one hour after blood collection. Storage of blood samples for more than one hour before analysis may cause underestimation of cell count.

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Handling and storage
For handling and storage of ChemoMetec instruments and 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 and cassettes refer to the corresponding product documentation and the NucleoCounter® NC-100™ 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-100™ system is FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE. The results presented by the NucleoCounter® NC-100™ system depend on correct use of the reagents, cassettes and the NucleoCounter® NC-100™ instrument and might depend on the type of cells being analyzed. Refer to the NucleoCounter® NC-100™ 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
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