910-0200

Reagent C

Package Insert

0.5 Litre

Contents

Container with 0.5 Litre of Reagent C

Application

The lysis buffer, Reagent C, is a part of the NucleoCounter® system. Reagent C is intended for the lysing of dispersed cells in suspension, with the intention of making the cell membrane permeable. It is generally applicable for the lysing of dispersed mammalian cells, somatic cells in milk and certain strains of yeast cells.

Principle

The addition of Reagent C to a sample containing dispersed cells, forming a lyzate solution, has the purpose of permeabilizing the cell wall and membranes, rendering the DNA of the cells susceptible to staining with propidium iodide. Also the addition of Reagent C has the purpose of diluting the sample and maintaining conditions which are suitable for the fluorescent detection of propidium iodide stained DNA.

For analysis approximately 60 µl of cell lyzate is aspirated into a NucleoCassette by pressing down the piston of the Cassette. Then the loaded Cassette is immediately placed in the NucleoCounter instrument and the "Run" button is activated. The fluorescent dye, propidium iodide, is immobilized in the first three lanes of the cassette flow system. As the cell lyzate is loaded into the NucleoCassette and transported through the flow system towards the measuring chamber, the immobilized propidium iodide is dissolved and mixed with the lyzate. Propidium iodide intercalates with DNA. Due to the fluorescent nature of propidium iodide it absorbs green light (536nm) and then emits red light (623nm), which is utilized for the detection of the stained DNA. The actual cell count is performed in the measurement chamber (the clear window) of the Cassette using the integrated fluorescence microscope of the NucleoCounter in combination with an automatic image analysis.

Procedure

Place the container in the container holder (if such is used). Remove the cap of the container. Mount the optional liquid dispenser (Dispensette® III , Brand GmbH) on the container. Handle the dispenser according to the instructions from the manufacturer. If pipetting Reagent C it is recommended that a suitable portion (e.g. 100 ml) is decanted into a clean container with replaceable sealing cap and that Reagent C is aspirated from that container to avoid possible contamination of the bulk solution.

With respect to the proposed procedure for analysing a cell sample, please refer to the Use's Guide for the NucleoCounter and/or the appropriate Application Note.

Stability

For sealed containers refer to the expiry date on the label of the container.

After breaking the seal of the container Reagent C expires after 12 months (room temperature). During this period the cap must be mounted on the container while not in use. Alternatively a dispensing unit must be mounted on the container and operated at least once a week to prevent reagent crystallization. It is recommended that the dispenser be cleaned in pure water before

prolonged storage.

Storage

Store Reagent C at room temperature and never above 40°C.

Safety information According to current legislation Reagent C is not regarded as a health or environmental hazard. It is not required to attach any hazard statements, hazard pictograms or risk/safety phrases to this reagent. However contact with skin and eyes should be avoided. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin wash immediately with plenty of water. Also refer to the Material Safety Data Sheet (MSDS), which is available on request.

Disposal of Waste

After use the Reagent C should be disposed of according to national or local laws and regulations regarding the nature of the mixture it contains.