910-0100 Reagent \$100 910-0101 5 Litres / 500 mL

Contents

Container with 5 Litres (910-0100) of Reagent S100 / 500 mL of Reagent S100 (910-0101)

Application

The lysis/dilution buffer, Reagent S100, is part of the NucleoCounter® SP-100™ system, which is comprised of the Reagent S100, the SP1-Cassette and the NucleoCounter SP-100. An optional part of the system is the SemenView™ software, which offers a variety of advantages to the user. The NucleoCounter SP-100 system is designed to count the total number of sperm cells in a sample of animal semen.

Principle

In order to count the total number of sperm cells in an ejaculates or other suspension of sperm cells, a sample containing the sperm cells is first treated with a lysis/dilution buffer called Reagent S100. The addition of Reagent S100 buffer has two purposes. First of all the Reagent S100 is used for disruption of the plasma membranes, rendering the nuclei of the sperm cells susceptible to staining with propidium iodide. Secondly, the reagent is used for dilution of the semen sample. For boars semen it is recommended to dilute the ejaculate 201 times by adding 50 μ l of semen to 10 ml of Reagent S100.

Approximately 60 µl of the diluted sample is aspirated into the SP1-Cassette by pressing down the piston of the cassette. Then the loaded SP1-Cassette is immediately placed in the NucleoCounter SP-100 and the "Run" button is activated. The fluorescent dye, propidium iodide, is immobilized in the first three lanes of the cassettes flow system. As the sperm cell is loaded into the SP1-Cassette and transported through the flowsystem towards the measuring chamber, the immobilized propidium iodide is dissolved and mixed with the diluted semen sample. Propidium iodide intercalates with DNA and stains the nuclei of the cells. Due to the fluorescent nature of propidium iodide it absorbs green light and then emits red light, which is utilized for detection of the stained nuclei. The actual cell count is performed in the measurement chamber (the clear window) of the SP1-Cassette using the NucleoCounter SP-100 with its integrated fluorescence microscope in combination with an automatic image analysis.

Procedure

For the 5 L container: Place the container in the container stand. Unscrew the screwcap of the container. Place the sample cup holder (if such is used) on the container. Mount the dispenser (Dispensette® III from Brand GmbH) on the container. When mounted correctly the dispenser will keep the sample cup holder in a fixed position. Adjust the position of the sample cup holder so that it is right below the discharge tube of the dispenser. Handle the dispenser according to the instructions from the manufacturer.

For the 500 ml container: This can be used with a dispenser but not with the sample cup holder.

With respect to the proposed procedure for analysing a sample of animal semen, refer to the User's Guide for the NucleoCounter SP-100 and the Application Notes.

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

After breaking the seal of the container the Reagent S100 will expire after 26 weeks. During this period the lid must be kept on the container or the dispensing unit must be mounted on the container.

Storage Store the container with the Reagent S100 at room temperature but never above 40°C.

Safety information

According to current legislation the Reagent S100 is not regarded as a health or environmental hazard. It is is not required to attach any hazard statements, hazard pictograms or risk/safety phrases to this reagent. However, the pH of Reagent S100 is relatively high (approximately 12,6). Therefore, avoid contact with skin and eyes. 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 MSDS, which is available on request.

Disposal of Waste

After use the Reagent S100 should be disposed of according to national or local laws and regulations regarding the nature of the mixture it contains.