Thermostatic device – working instructions

Thermostatic device for heating up samples for detection of inhibitor substances in cow, sheep, goat milk etc at constant temperature, which could be changed using PC.

Working voltage – 12 V-16V
Using pulse power supply 110-240V

Working with the thermostatic device:

1. Connect the output of the pulse power supply to the thermostatic device.
2. Connect the pulse power supply to the network.
3. The green and red LED on the front panel of the thermostatic device light up
4. In dependence of the outer temperature, after appr. 5 minutes the red LED lights off, which indicates that the thermostatic device has reached the set temperature and is ready for work. When Eclipse 50 inhibitor test is used - temperature is factory set to 65^{o} C
5. In case you need to switch off the thermostatic device, simply disconnect the power supply.

Note: when working with accumulator power supply use the cable with the plug suitable to be connected to the lighter, which is part of the set.

ECLIPSE 50

Consumable for the thermostatic device for detection of inhibitors in the milk

Working instructions

Test for the detection of inhibitors and antibiotics in milk. This is a rapid and simple method to check whether milk contains antibiotics in a concentration exceeding the Maximum Residue Limits (MLR).

PRINCIPLE

Based on the inhibition of microbial growth. The kit is presented in a microplate format. Each well contains the agar medium spread with *Bacillus tearothermophilus* spores plus a pH indicator.
When the plate is incubated at 65ºC (into the thermostatic device), the spores germinate and grow, reducing the pH of the medium, which will shift the initial colour from blue (purple) to green-yellow.
If milk samples contain an antibiotic concentration higher than the detection limit of the test, the microbial growth and acid production is inhibited. There is no acid production and no colour change is observed.

KIT COMPONENTS

- 96 individual tests microtiter plate. Each well contains growth medium spread with Bacillus *stearothermophilus* spores.

- Adhesive foil to seal the plate.
- Optional: An additional plastic frame

STABILITY AND STORAGE

The kit components should be stored at 2-8 ºC in darkness. Kit keeps stability for months in optimal storage conditions.

SAFETY INSTRUCTIONS

It is recommended a person with laboratory practice to work with this set. Wear suitable working cloths and protect the skin from contacts with reagents. Do not swallow!

NOTES

- It is recommended to apply a **negative control** (antibiotic-free milk) and a **positive control** containing a high concentration of antibiotic (for example penicillin G 10 mg/L) for determining the optimal incubation time. If goat samples are analysed, the negative control must be goat´s milk, without inhibitors. It should be used a clean pipette tip for each sample.
- This test is extremely sensitive to antibiotics and other antibacterial substances such as detergents and disinfectants. Any contamination with these substances should be prevented.
- Plates should be kept closed in the plastic bag to avoid drying out of the wells and stored at 2-8ºC.
- Natural inhibitors occurs in milk at low concentration which do not interfere with the test results, but inhibitor substances increase in colostrum, at the end of breeding period and in the case of mastitis. In those cases the results could be altered.
- If samples containing preservatives are to be analysed, the time of incubation could be extended for some minutes.

TEST PROCEDURE FOR COW AND GOAT´S MILK

1. Cut the metallic foil around the wells to be used. Take care not to take off the foil on the remaining part of the plate as this will lead to drying out of the wells. It is advised to use strips of 8 wells for each analysis although it is also suitable for individual test.

2. Remove the strips to be used from the plastic frame. Simply press up the strip with the fingertip, then it will pop up for easy removal by hand. The remaining tests in the plate should be immediately kept closed in the plastic bag to avoid drying out of the wells and should be stored at 6-15ºC.

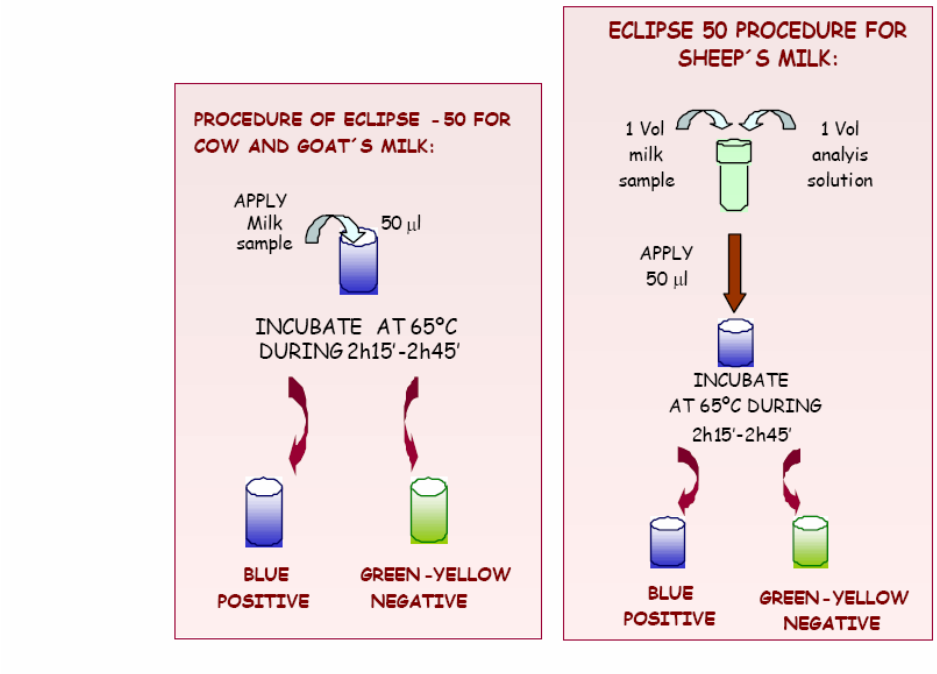
3. Place the wells to be used on another white plastic frame. Peel off the adhesive foil on the plate and apply **50 ml** of milk sample in each well. It is advisable to use automatic micropipettes.

4. Seal carefully the plate or the strips with the adhesive foil (or “Scotch tape” for one or more wells) and float upright in the thermostatic device pre-warmed at **65ºC**. Be

sure that the wells are perfectly sealed. If water drops into the wells during incubation, wrong results could be obtained.

5. Incubate for 15 min beyond the time in which the negative control sample has reached the green-yellow colour (total time approx. 2h15'-2h45'). For a higher sensitivity read the result when the negative control sample reaches the yellow-green colour. If the negative control has not changed to green-yellow, continue the incubation for some minutes.

6. Read the results from a side of the well, better than the bottom. A **green-yellow** colour (negative) indicates the absence of antibiotics in the tested milk sample. A **purple** colour indicates the presence of antibiotics (positive). A green-blue colour (questionable) indicates the presence of antibiotics in a concentration close to the detection limit. In this case it is recommended remaking the analysis.



PROCEDURE FOR THE ANALYSIS OF SHEEP´S MILK (only for ECLIPSE 50ov)

IMPORTANT! This procedure is developed for sheep's milk analyses. Before usage of ECLIPSE 50ov it is recommended to read above instructions. Not suitable for cow milk.

1. Dilute 1/100 the **analysis solution.** For example, add 1 ml of the stock solution to 99 ml of distilled water. The diluted solution is stable for 3-4 weeks under refrigeration.

2. Mix thoroughly one volume of the milk sample with one volume of the **analysis solution** (diluted 1/100).

The sample is now ready to apply.

3. Remove the adhesive foil on the plate and apply **50 ml** of the diluted milk samples into each well.

4. Seal carefully the plate with a new adhesive foil and float upright in the thermostatic device pre-warmed at **65ºC** (approximately 2h15' - 2h45').

5. Incubate for 15 min beyond the time in which the negative control sample has reached the yellow colour (approx. 2h15'- 2h45'). If the negative control has not changed to green-yellow, continue the incubation for some minutes.

6. Read the results from the plate bottom side. A green-**yellow** colour (negative) indicates the absence of antibiotics in the tested milk sample. A **purple** colour indicates the presence of antibiotics (positive). A green colour (questionable) indicates presence of antibiotics in a concentration close to the detection limit. In this case a repetition of the analysis is recommended.

Attention: Intensity of the colors reveived for positive and negative resultas are one and the same as those with ECLIPSE 50 for cow milk.

Detection limit of the ECLIPSE test for several inhibitors (mg/ml) in cow´s milk.

ECLIPSE 50	NEGATIVE	POSITIVE
PENICILLIN G	0,002	0,004
AMPICILLIN	0,003	0,005
AMOXICILLIN	0.003	0.005
OXACILLIN	0,005	0,025
CLOXACILLIN	0,025	0,04
CEPHALEXIN	0.025	0.075
CEPHAPIRINE	0.005	0.008
SULFATHIAZOLE	0,02	0,075
SULFAMETHAZINE	0,1	0,2
SULFANILAMIDE	0,1	0,6
OXYTETRACYCLIN	0,05	0,15
TETRACYCLIN	0,05	0,15
ERYTHROMYCIN	0,2	0,4
TYLOSIN	0,02	0,1
NEOMYCIN	<0.500	0.80