ECLIPSE 50

Test para la detección de substancias antibacterianas en leche

Test for the detection of inhibitory substances in milk
INTENDED USE

ECLIPSE is a 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

ECLIPSE is based on the inhibition of microbial growth. The kit is presented in a microplate format. Each well contains the agar medium spread with Bacillus stearothermophilus spores plus a pH indicator. When the plate is incubated at 65°C (into an incubator or water-bath), 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

If some anomaly is observed, please contact with Z.E.U.-INMUNOTEC or your regular distributor.

STABILITY AND STORAGE

The kit components should be stored at 2-8 °C in darkness. Kit keeps stability for 6 months in optimal storage conditions.

ADDITIONAL MATERIAL (NOT PROVIDED)

- Micropipettes
- Water bath or incubator at 65°C
- Milk free of antibiotics

SAFETY

Good laboratory practice should be employed when using this kit. Safety clothing should be worn and skin contact with reagents avoided. Do not ingest. A SAFETY DATA SHEET is available by request from your distributor.

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 2-8°C.
- 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 (e.g. Azidiol).

ECLIPSE is an in vitro diagnostic kit for the screening of milk samples. In analysis implicating legal processes, the results should be reevaluated with an official reference method. ZEU-INMUNOTEC, S.L. do not assume any legal responsibility.
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 µl 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 a water-bath or incubator prewarned 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 OF ECLIPSE - 50 FOR COW AND GOAT’S MILK:**

- **APPLY**
  - Milk sample

  50 µl

- **INCUBATE AT 65ºC DURING 2h15’-2h45’**

- BLUE POSITIVE

- GREEN-YELLOW NEGATIVE

**ECLIPSE 50 PROCEDURE FOR SHEEP’S MILK:**

- **1 Vol milk sample**

- **APPLY**
  - 50 µl

- **INCUBATE AT 65ºC DURING 2h15’-2h45’**

- BLUE POSITIVE

- GREEN-YELLOW NEGATIVE
PROCEDURE FOR THE ANALYSIS OF SHEEP’S MILK (Only for ECLIPSE 50ov kit)

Important! This procedure has been developed for sheep’s milk analysis. Before using ECLIPSE 50ov for the first time it is recommended to read the general indications written above. It is not suitable for cow’s milk.

1. Dilute 1/100 the analysis solution (100 X). 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 l of the diluted milk samples into each well.

4. Seal carefully the plate with a new adhesive foil and float upright in a water-bath or incubator prewarmed at 65°C (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.

Caution: Intensity of the obtained colours for the positive and negative results are not the same as the ECLIPSE 50 colours for cow’s milk.

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

<table>
<thead>
<tr>
<th>ECLIPSE 50</th>
<th>NEGATIVE</th>
<th>POSITIVE</th>
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<tbody>
<tr>
<td>PENICILLIN G</td>
<td>0.002</td>
<td>0.004</td>
</tr>
<tr>
<td>AMPICILLIN</td>
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<td>0.005</td>
</tr>
<tr>
<td>AMOXICILLIN</td>
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</tr>
<tr>
<td>OXACILLIN</td>
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<td>CLOXACILLIN</td>
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<tr>
<td>CEPHALEXIN</td>
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<tr>
<td>CEPHAPIRINE</td>
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<tr>
<td>SULFATHIAZOLE</td>
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<tr>
<td>SULFAMETHAZINE</td>
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<tr>
<td>SULFANILAMIDE</td>
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<tr>
<td>NEOMYCIN</td>
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OTROS PRODUCTOS PARA LA INDUSTRIA LECHERA

- **Kit RC** para la detección y cuantificación de leche de vaca y cabra en leche o queso de oveja mediante la técnica ELISA.

- **Kit IC** para la detección rápida de leche de vaca y de cabra en leche o queso de oveja. ¡5 minutos!

- **CALOKIT** para la detección de calostro en leche. Evite la precipitación de proteinas en sus intercambiadores de calor. Mejore los rendimientos queseros y evite los olores y sabores extraños en sus quesos.

- **PROTEON** para la detección de proteínas vegetales en leche y productos lácteos mediante la técnica de ELISA.

PRODUCTS FOR THE DAIRY INDUSTRY

- **RC test**: ELISA test for the detection and quantification of cow’s or goat milk in sheep’s milk or cheese.

- **IC test**: Immunochromatographic test for a rapid detection of cow’s or goat milk in sheep’s milk or cheese). Only 5 minutes!

- **CALOKIT**: ELISA test for colostrum detection in milk. Avoid proteins precipitation in the heat transfers. Improve the cheese yields and prevent strange smell and taste in cheese.

- **PROTEON**: ELISA test for detection of vegetable proteins in milk and dairy products.

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