



ECLIPSE 50

ZE/E50/96i

ZE/E50/288i

**Test for detection of inhibitory
substances in milk**

**Test para la detección substancias
antibacterianas en leche**

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SCOPE

ECLIPSE is a qualitative test, supplied in a flexible and handy format, for detection of antibiotics and inhibitors in raw, heated or powder milk from cow, sheep or goat.

PRINCIPLE

ECLIPSE is a test based on the inhibition of microbial growth. It is supplied in a microtiter plate format where each well contains agar medium spread with *Geobacillus stearothermophilus* spores and a pH indicator.

When the plate is incubated at 65°C, spores germinate and cells grow producing acid and changing the agar pH. Variations of pH will produce changes of the agar color from blue (purple) to yellowish.

When milk samples contain antibiotic at higher concentrations than the detection limit, microorganisms will not grow and neither color changes will be observed.

KIT COMPONENTS

	ZE/E50/96i	ZE/E50/288i
Individual tests	96	288
Microtiter plates	1	3
Adhesive foil	1	3
Kit instructions	yes	yes
Product certificate	yes	yes

STABILITY AND STORAGE

The kit components should be stored at 4-12 °C and protected from light. This kit is provided with a minimum shelf life of 9 months. See the kit expiring date on package.

ADDITIONAL MATERIAL (NOT PROVIDED)

- ✓ Micropipettes.
- ✓ Heater (FX incubator, ref: ZE/FX) or oven at 65°C.
- ✓ Negative control (sample without antibiotics) (sheep milk ref. ZE/LPO200; goat milk ref. ZE/LPC200; cow milk ref. Merck 1.15363.0500, Scharlab 06-019).
- ✓ Positive control - freeze dried Penicillin G (ref. ZE/PG5).

SAFETY

Good laboratory practices are recommended when using this kit. A Material Safety Data Sheet (MSDS) is available from your local distributor or ZEU-INMUNOTEC by request.

NOTES

- ✓ A negative control sample (without antibiotics) must be used to determine the optimal incubation of the assay in each run. See negative control references for cow, sheep and goat milk under "Additional material" paragraph.

- ✓ Testing a positive control sample is also recommended.
- ✓ A new pipette tip should be used 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.
- ✓ Although natural inhibitors contained in milk do not interfere with the test results, samples from colostrum, milk from the end of the breeding period, and mastitis milk have high concentration of these inhibitors and can alter the results.
- ✓ Please, contact ZEU-INMUNOTEC for the analysis of samples containing preservatives (i.e. Azidiol).

TEST PROCEDURE (Flowchart Procedure on page 8)

- 1.- Cut the adhesive foil sheet covering the wells and split the strips to be used by pressing up from the bottom of the wells.
The foil covering the remaining wells should not be removed and the wells stored immediately at 4-12°C, to prevent the wells from drying up.
- 2.- Remove the adhesive foil covering the plate/wells, and add 50 µl of sample per well, including a negative control sample.
- 3.- Seal carefully the strips with adhesive film and incubate at 65°C.
The incubation should be stopped when the negative control sample has turned to yellow (approximately 2h15' - 2h45'). See the product certificate and use the incubation time shown as reference.
- 4- When the negative control sample has changed to yellowish color, turn the plate upside down to remove the remaining sample. Wash the wells with distilled water by filling the wells up. Empty the wells by turning the plate upside down on top of an absorbent paper to remove the excess of water. Repeat the washing step 2 to 3 times.
- 5.- Turn the wells upside down and read the results comparing each sample with the negative control well. Identify the colors as follows (see the color card and page 8).
 - A yellow color indicates the absence of antibiotics in the tested milk sample (negative) .
 - A blue color indicates the presence of antibiotics (positive)
 - Colors ranging from yellow to blue indicate presence of antibiotics in a concentration close to the detection limit. Analysis should be repeated when doubtful.



ECLIPSE is an *in vitro* diagnostic kit for antibiotics screening in milk. 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

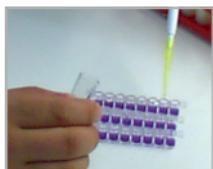
Limits of detection (LOD) ($\mu\text{g/L}$ or ppb) of ECLIPSE 50 for different inhibitors in cow milk:

INHIBITOR	LOD ($\mu\text{g/L}$)
b-LACTAMS	
Amoxicillin	4
Ampicillin	4-5
Cefalexin	75
Cefapirin	8
Cefalonium	20
Ceftifur	100
Cloxacillin	35
Oxacillin	25
Penicillin G	3-4
TETRACYCLINES	
Doxycycline	100
Oxitetracycline	100
Tetracycline	100
MACROLIDES	
Erythromycin	400-800
Tylosin	80-100
Spiramycin	>400
AMINOGLYCOSIDES	
Streptomycin	2.000
Gentamycin	400
Neomycin	1.500
Kanamycin	>2.000
SULFONAMIDES	
Sulfadiazine	100
Sulfametazine	150
Sulfamethoxypyridazine	100
Sulfanilamide	600
Sulfamethoxazole	100
Sulfathiazole	100
LINCOSAMIDES	
Lincomycin	150-300
OTHERS	
Chloranphenicol	5.000

Please contact ZEU-INMUNOTEC or your local distributor for information on limit of detection of other species

FLOWCHART PROCEDURE

ESQUEMA DEL PROCEDIMIENTO



Add 50 μ l of milk

Añadir 50 μ L de leche



Incubation at 65°C up to the negative control has turn to yellow.

Incubación a 65°C hasta que el control negativo haya virado a amarillo

Wash and read from the bottom the the wells

Lavar y leer desde el fondo del pocillo

