ECLIPSE 3G

ZE/E3G/96i
ZE/E3G/288i

Test for detection of inhibitory substances in milk

Test para la detección de sustancias antibacterianas en leche

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SCOPE

ECLIPSE is a qualitative test, supplied in a flexible and handily 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 65ºC, spores germinate and cells grow producing acid and changing the agar pH. Variations of pH will produce changes of the agar colour from blue (purple) to yellowish. When milk samples contain inhibitors at higher concentrations than the detection limit, microorganisms will not grow and neither colour changes will be observed.

KIT COMPONENTS

	ZE/E3G/96i	ZE/E3G/288i
Individual tests	96	288
Microtiter plates	1	3
Adhesive film	1	3
Kit instructions	A	A
Product certificate	A	A

ADDITIONAL MATERIAL (NOT PROVIDED)

Micropipettes.

Heater (FX incubator, ref: ZE/FX) or oven at 65ºC.

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 4-12ºC, to prevent the wells from drying up.

2.– Remove the adhesive foil covering the wells/plate, and add 100 µL of sample per well, including a negative control sample.

3.- Seal carefully the wells with adhesive film and incubate at 65ºC.

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 colour, 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.– Results

Visual reading: Turn the wells/plate upside down and read the results comparing each sample with the negative control well. Identify blue wells as positive and yellow wells as negative (see the colour card and page 8). Colours ranging form yellow to blue indicate presence of antimicrobials in a concentration close to the detection limit. Analysis should be repeated when doubtful.

Photometric reading: Read the wells/plate at 590nm (filter 1) and at 650nm (filter 2). The assay must be stopped when the difference of absorbance of negative control (AN 590nm - AN 650nm) is between 0.2 and 0.5 units. Samples with results mayor or equal to those obtained for the samples used as negative control plus 0.2 will be positive.

POSITIVE: AM 590nm - AM 650nm ≥ AN 590nm - AN 650nm + 0.2

AM: Sample absorbance

AN: Negative control absorbance

Note: The above procedure is only applicable when the difference of absorbance at 590nm - 650nm of the negative control sample is between 0.2 and 0.5.

If the value is higher than 0.5, incubate the wells again at 65ºC to obtain the difference of absorbance between 0.2 and 0.5 (approximately 10 - 15 min).

ECLIPSE is an *in vitro* diagnostic kit for antibiotics screening in milk. In analysis implicating legal processes, the results should be revaluated with an official reference method. ZEU-INMUNOTEC, S.L. do not assume any legal responsibility.

To the best of our knowledge, the information contained herein is accurate and complete. However, nothing herein shall be construed to imply any warranty or guarantee.

Limits of detection (LOD) of ECLIPSE 3G in cow milk:

LÍMITES DE DETECCIÓN (LOD) DE ECLIPSE 3G EN LECHE DE VACA

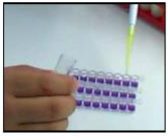
INHIBITOR	LOD (µg/L)
β-LACTAMS	
Amoxicillin	3
Ampicillin	3
Cefalexin	60
Cloxacillin	20
Oxacillin	10
Penicillin G	2
TETRACYCLINES	
Doxicycline	100
Oxytetracycline	50
Tetracycline	100
SULFONAMIDES	
Sulfadiazine	100
Sulfametazine	150
Sulfamethoxypyridazine	100
Sulfathiazole	50
MACROLIDES	
Erythromycin	200
Tylosin	40
AMINOGLYCOSIDES	
Streptomycin	2.500
Gentamycin	>1.000
Neomycin	>1.000
Espectinomicine	>2.500
LINCOSAMIDES	
Lincomycin	>150
OTHERS	
Chloramphenicol	5.000

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

Por favor, contacte con ZEU-INMUNOTEC o su distribuidor local para obtener información sobre los límites de detección en otras especies.

FLOWCHART PROCEDURE

ESQUEMA DEL PROCEDIMIENTO



Add 100 L of milk

Añadir 100 L de leche

Incubation at 65ºC up to the negative control has turned to yellow.

Incubación a 65ºC hasta que el control negativo haya virado a amarillo.

Wash and read:
Visual reading from the bottom of the wells
Photometrical reading at 590 and 650 nm.

Lavar y leer:
Lectura visual desde el fondo del pocillo
Lectura fotométrica a 590 y 650 nm