



EXPLORER

Ref. ZE/EX96i
ZE/EX480i

*Test for detection of inhibitory substances
in foods*

*Test para la detección de sustancias
antibacterianas en alimentos*

ZEU-INMUNOTEC S.L.
María de Luna 11, Nave 19 • 50018 Zaragoza (SPAIN)
Tel.: 34 976 73 15 33 • Fax: 34 976 52 40 78 • info@zeu-inmunotec.com
www.zeu-inmunotec.com

SCOPE

EXPLORER is a test for detection of inhibitory substances in raw meat, liver, kidney, feed and eggs. It is a rapid and simple method to check whether the sample contains antibiotics at concentrations exceeding the Maximum Residue Limits (MRL).

PRINCIPLE

EXPLORER is based on the inhibition of microbial growth. The kit is supplied in a 96-well microplate, where each well contains agar medium spread with a bacillus thermophile spores plus a redox indicator.

When the test is incubated at 65°C, bacteria cells grow modifying the redox potencial of the medium and changing the agar colour from blue to orange. If samples contain antibiotics at concentrations above the limit of detection of the kit, microorganisms will not grow neither colour changes will be observed.

KIT COMPONENTS

| | ZE/EX96i | ZE/EX480i |
|---------------------|-----------------|------------------|
| Individual tests | 96 | 480 |
| Microtiter plates | 1 | 5 |
| Adhesive sheet | 2 | 10 |
| Product certificate | yes | yes |

STABILITY AND STORAGE

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

ADDITIONAL MATERIAL (NOT PROVIDED)

- ✓ Micropipettes.
- ✓ Heater (FX incubator, ref. ZE/FX), water bath or oven at 65°C.
- ✓ Laboratory balance.
- ✓ Magnetic stirrer or roller.
- ✓ Liver Solution - EX (diluting solution for liver) (ref. ZE/EX/SOLD8)
- ✓ Negative control sample for meat and liver (sample without antibiotics) (ref. ZE/EX/CN1/1, ZE/EX/CN1/5).
- ✓ Negative control sample for eggs (sample without antibiotics) (ref. ZE/EX/CN2)
- ✓ Negative control sample for feed: PBST (see composition in Sample preparation).
- ✓ Positive control sample - freeze dried Penicillin G (ref. ZE/PG5) - *optional*.

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) should be used to determine the optimal incubation of the assay in each run. See negative control references for meat, liver and eggs under “Additional material” paragraph. PBST should be used as negative control for feed (see composition in “Sample preparation”).
- ✓ Testing a positive control sample is recommended.
- ✓ An additional solution is needed for liver samples. See “Sample preparation” or contact ZEU-INMUNOTEC for more information.
- ✓ Mince meat and marinade, cured or smoked meat products might inhibit EXPLORER bacteria, because of their particular physical or chemical characteristics. This inhibition of the test would not necessarily be related to antibiotic or sulfonamide residues found in the samples.



- ✓ Samples should be analysed immediately upon receipt or stored under refrigeration for a maximum of 24 hours or frozen for longer periods of time.
- ✓ Calibrated pipette must be used for adding the sample.
- ✓ A new pipette tip must be used for each sample.
- ✓ Glass or plastic tubes resistant to heat of about 1.5 cm diameter are recommended for sample preparation.
- ✓ This test is extremely sensitive to antibiotics and other antibacterial substances such as detergents and disinfectants. Any contamination with these substances should be prevented.

SAMPLE PREPARATION

Fresh meat samples

1. Weigh 3 ± 0.5 g of lean meat without adipose or conjunctive tissue and cut it into small pieces.
2. Place the meat tissue in a glass or plastic tube (of about 1.5 cm diameter) resistant to heat, and seal.
3. Heat for 3 ± 0.5 min. at 100°C .
4. Cool down by dipping the tube in cold water.
5. Squeeze the meat piece using a pair of tweezers to obtain as much juice as possible.
6. Place the juice in an eppendorf and centrifuge for 3 min. at 2000 g. Alternatively, filter the meat juice using a $0.45 \mu\text{m}$ filter.
7. Add 100 μL of the supernatant to the well.

Liver samples

1. Weigh 10 ± 0.5 g of liver tissue and cut it into small pieces.
2. Place the liver tissue in a glass or plastic tube (of about 1.5 cm diameter) resistant to heat, and seal.
3. Heat for 12 ± 0.5 min. at 100°C .
4. Cool down by dipping the tube in cold water.
5. Place the juice in an eppendorf and centrifuge for 3 min. at 2000 g. Alternatively, filter the juice using a $0.45 \mu\text{m}$ filter.
6. Take 200 μL of the juice and mix with 100 μL of Liver Solution - EX (ZE/EX/SOLD8).
7. Add 100 μL of the mixture to the well.

Kidney samples

1. Weigh 5 ± 0.5 g including medulla and cortex.
2. Place the sample in a glass or plastic tube resistant to heat of about 1.5 cm diameter and seal.
3. Heat for 4 ± 0.5 min. at 100°C .
4. Cool down by dipping the tube in cold water.
5. Place the juice in an eppendorf and centrifuge for 3 min. at 2000g. Alternatively, filter the juice using a $0.45 \mu\text{m}$ filter.
6. Add 100 μL of the supernatant to the well.

Feed samples

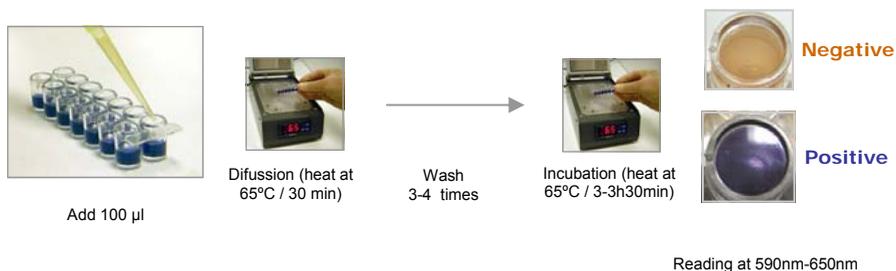
1. Weigh 1 ± 0.1 g of ground feed sample, place in a clean tube and seal.
2. Add 20 mL of PBST previously preheated (approximately 40°C).
PBST (5 L): 45 g of NaCl, 80 mL of K_2HPO_4 0.5 M, 16.7 mL KH_2PO_4 0.5 M, 5 mL Tween 20, adjust the pH to 7.4 (with K_2HPO_4 or KH_2PO_4).
3. Homogenise the mixture for at least 30 min. using a magnetic stirrer or a roller until the sample is fully dissolved.
4. Centrifuge for 15 min. at 2000 g. Alternatively, filter the mixture using a 0.45 μ m filter.
5. Add 100 μ L of the supernatant to the well.

Egg samples

1. Place white and yolk into a clean container and beat to obtain an homogeneous fluid.
2. Take 1 mL of egg and add 3 mL of distilled water in a glass or plastic tube resistant to heat of about 1.5 cm diameter, seal.
3. Heat the sample for 12 ± 0.5 min. at 100°C.
4. Mix vigorously for 20-30 seconds to avoid sample coagulation. Let the sample to cool down at room temperature.
5. Add 100 μ L of the mixture to the well.

TEST PROCEDURE

1. Cut the foil sheet covering the wells and split the strips to be used by pressing up from the bottom of the wells. Although single wells can be used, it is recommended to use strips of 8 wells each time.
2. Peel off the adhesive foil covering the plate and add 100 μ l of each sample per well. Add also, 100 μ l of negative control sample per well. See negative control sample for each matrix in "Additional Material". Make sure the remained wells are perfectly sealed and stored in a plastic bag at 4-12°C to prevent the agar from drying up.



3. Seal the plate/wells carefully with an adhesive sheet and heat the sample at 65°C for 30 minutes to allow the sample to diffuse through the well.
4. Empty the wells/plate by turning the plate upside down and wash the plate/wells with distilled water by adding 300 µl/well of distilled water when using a micropipette, or filling the well up when using a squeeze bottle. Empty the wells/plate by turning the plate upside down on top of an absorbent paper to remove the excess of water. Repeat the washing step 3 to 4 times.
5. Seal the wells/plate carefully with an adhesive sheet and incubate at 65°C until the negative control sample has turned orange (approximately 3h-3h30min). See the product certificate and use the incubation time shown as reference.
6. Results interpretation:
 - ✓ Visual reading of results: Turn the plate upside down and identify the blue wells as positive and the orange as negative.
 - ✓ Photometric reading: Read the wells/plate at 590 nm (Filter 1) and at 650 nm (filter 2). Results will be interpreted as the difference between the values of the two readings. The assay must be stopped when the difference of absorbance of negative control (AN 590nm - AN 650nm) is between 0.15 and 0.25. Samples with results \geq to those obtained for the samples used as negative control plus 0.15 will be positive.

POSITIVE: $AM\ 590nm - AM\ 650nm \geq AN\ 590nm - AN\ 650nm + 0.15$

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 0.15 and 0.25.

* **EXPLORER** is an *in vitro* diagnostic kit for the screening of food samples. In analysis implicating legal processes, the results should be reevaluated with an official reference method. ZEU-INMUNOTEC does not assume any legal responsibility.



KITS FOR AGROFOOD DIAGNOSTICS

- **RC:** ELISA test for quantification of cow's or goat's milk in sheep's milk or cheese.
- **IC:** Immunochromatographic test for qualitative detection of cow or goat's milk in sheep's milk or cheese.
- **CALOKIT:** ELISA test for quantification of IgG's in milk (detection of colostrum in milk).
- **PROTEÓN:** ELISA test for detection of allergens in food.
- **ECLIPSE:** Microbiological test for screening of inhibitory substances in milk.
- **SCREENING PLUS:** Microbiological test for detection of aminoglycosides and macrolides in milk.
- **EXPLORER:** Microbiological test for detection of inhibitory substances in raw meat, liver, kidney, feed and eggs.
- **TOXILINE-DSP:** Enzymatic test for quantification of marine toxins responsible for Diarrhetic Shellfish Poisoning (DSP).
- **MICROFAST-E.COLI 0157:** Immunochromatographic test for detection of Escherichia Coli O157 in food.
- **μ-Cystest:** Enzymatic test for detection of microcystins in water.

KITS DE DIAGNÓSTICO ALIMENTARIO Y VETERINARIO

- **RC:** Test ELISA para la cuantificación de leche de vaca y cabra en leche o queso de oveja.
- **IC:** Test inmunocromatográfico para la detección rápida de leche de vaca y de cabra en leche o queso de oveja.
- **CALOKIT:** Test ELISA para la cuantificación de IgG's en leche (detección de calostro en leche).
- **PROTEÓN:** Test ELISA para la detección de alérgenos en alimentos.
- **ECLIPSE:** Test microbiológico para la detección de inhibidores en leche.
- **SCREENING PLUS:** Test microbiológico para la detección de inhibidores en leche, optimizado para aminoglicósidos y macrólidos.
- **EXPLORER:** Test microbiológico para la detección de inhibidores en carnes frescas, hígado, riñón, piensos y huevo.
- **TOXILINE-DSP:** Test enzimático para la detección y cuantificación de toxinas diarreas (DSP) en moluscos.
- **MICROFAST-E.COLI 0157:** Test inmunocromatográfico para la detección de *Escherichia coli* 0157 en alimentos.
- **μ-Cystest:** Test enzimático para la detección de microcistinas en agua.