Biorex food diagnostics

CHLORAMPHENICOL FAST ELISA

PRODUCT CODE: BXEFB03A

QUALITY MANAGEMENT SYSTEM
ISO 13485 CERTIFIED COMPANY

BXEFB03A
96 TESTS (12x8 STRIP WELLS)
STORE AT 2-8°C
INSTRUCTIONS FOR USE
FOR VETERINARY USE AND/OR USE IN THE ANALYSIS OF FOOD ONLY. Not for use in diagnostic procedures.

LOT Lot Number
REF Catalogue Number
Storage Temperature
Expiry Date (Year / Month)
Warning, Read Enclosed Documents
Instructions For Use
Manufactured By

CHLORAMPHENICOL FAST ELISA

Introduction
Enzyme-Linked Immunosorbent Assay for the Determination of chloramphenicol and chloramphenicol glucuronide in animal urine, shrimp/prawn, milk and honey samples. Not for use in human diagnostics.

Importance of Chloramphenicol determination
Chloramphenicol is a broad spectrum antibiotic which is predominantly active against the main pathogen gram negative bacteria occurring in a number of food producing animals. Chloramphenicol is an antibiotic not authorised for use in food-producing animals in the European Union. Antibiotic residues such as chloramphenicol in foods pose a serious threat to public health due to aplastic anaemia. Due to this risk the use of chloramphenicol is food producing animals is prohibited in many countries worldwide with zero tolerance limits being imposed. It is therefore essential that the presence of this drug is monitored in food products such as meat, milk, honey and seafood.

Samples
Shrimp/Prawn, Urine, Milk, Honey

Sample Preparation
Centrifugation and apply neat (urine, milk).
Dilution in water followed by solvent extraction, agitation, centrifugation, evaporation, reconstitution and apply (shrimp, honey)

Assay Time
45mins

Limit of detection

Shrimp/Prawn	0.05ppb
Urine	0.1ppb
Milk	0.1ppb
Honey	0.05ppb

Specificity

Chloramphenicol	100%
Chloramphenicol Glucuronide	>100%

Test Principle
The Chloramphenicol quantitative test is based on the principle of the enzyme-linked immunosorbent assay. A specific Chloramphenicol antibody is coated on the surface of a microtiter plate. Chloramphenicol containing samples or standards and an HRP conjugate ezyme is pipetted into the wells of the microtiter plate. The chloramphenicol contained in samples or standards and the HRP conjugate will compete with binding sites to the antibody coated microtitre plate wells. After an initial incubation step at room temperature for 30 minutes the wells are washed 4 times with diluted washing solution to remove unbound material. A substrate solution is added and incubated for 15 minutes, resulting in the development of a blue colour. The colour development is inhibited by the addition of a stop solution, and the colour turns yellow. The yellow colour is measured photometrically at 450 nm. The concentration of chloramphenicol is indirectly proportional to the colour intensity of the test sample.

Reagents Provided
The kit contains reagents for 96 determinations. They have to be stored at 2-8°C. Expiry data are found on the labels of the bottles and the outer package.

Code / Size	Component / Description
FB03-001 **FB03-002** **FB03-003** **FB03-004** **FB03-005** **FB03-006** (6 vials with 1mL each)	STANDARD A – 0.0 ng/ml STANDARD B – 0.05 ng/ml STANDARD C – 0.125ng/ml STANDARD D –0.5 ng/ml STANDARD E –1 ng/ml STANDARD F – 4 ng/ml Ready-to-use
FB03-018 (1 x 1ml)	**SPIKING SOLUTION**– Chloramphenicol 100ng/ml
FB03-008 (1x96 wells) 8×12/12×8 well per plate	**MICROTITER PLATE** consisting of 12 strips with 8 breakable wells each, coated with a chloramphenicol antibody.
WS-009 (1x20ml)	**WASH BUFFER:** Wash Buffer Solution (50X), Concentrate 20ml. If using full bottle add 980ml double de-ionised water and mix. Once diluted

DILUTE BEFORE USE!	the wash buffer solution is stable for 30days when stored at 2-8oC. (Detergent Tween-20)
ST-010 (1x11ml)	**STOP SOLUTION:** Colorless liquid in a clear vial with red screw cap. Diluted HCL acid solution. Ready to use as supplied.
SS-011 (1x13ml)	**SUBSTRATE SOLUTION:** Clear solution, ready-to-use.
FB03-012 (1x11ml)	**CONJUGATE:** Amber vial, (Chloramphenicol-HRP), ready-to-use.
1x	**PLATE COVER** To cover the strips during the incubation.
1x	**IFU** (Instruction Manual)

Materials required but not provided

Instrumentation

- 50, 100, 500 and 1000 µL-micropipettes
- 10,000 µL-pipette or glass pipette
- ELISA reader (450 nm)
- Centrifuge
- Ultra-Turrax homogeniser, blender
- Evaporator
- Vortex/Roller

Reagents

- Double distilled water
- Ethyl acetate
- n-Hexane

Safety Precautions

1. Do not smoke or eat or drink or pipet by mouth in the laboratory.
2. Wear disposable gloves whenever handling patient specimens.
3. Avoid contact of substrate and stop solution with skin and mucosa (possible irritation, burn or toxicity hazard). In case of contact, rinse the affected zone with plenty of water.
4. Handling and disposal of chemical products must be done according to good laboratory practices (GLP).

Handling and Storage Instructions

Full compliance of the following good laboratory practices (GLP) will determine the reliability of the results:

1. Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
4. Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
5. Use a separate disposable tip for each specimen to prevent cross-contamination.
6. All specimens and standards should be run at the same time, so that all conditions of testing are the same.
7. Do not mix components from different batches.
8. Do not use reagents after expiration date.
9. Check both precision and accuracy of the laboratory equipment used during the procedure (micropipets, ELISA reader etc.).

Sample Preparation

Urine

1. Centrifuge urine at 4500 g for 10 minutes.
2. Apply the supernatant directly to the microtiter plate.

Shrimps/Prawn

1. To 3 g sample with 3 mL double distilled water, add 6 mL ethyl acetate and agitate vigorously for 10 minutes (vortexing/rolling).
2. For phase separation centrifuge for 10 minutes at 3000 g (room temperature).
3. Transfer 4 mL of the upper ethyl acetate phase to a clean glass vial and evaporate the solvent at 50-70°C under a nitrogen or airstream to dryness.
4. Add 1 mL n-hexane to the residue.
5. Add 500 µL diluted wash buffer (1X) to the mixture and vortex for 1 minute.
6. For phase separation centrifuge for 10 minutes at 3000 g (room temperature).
7. Apply the lower aqueous phase to the microtiter plate.

   If an emulsion forms and there is difficulty pipetting from the lower layer the sample can be incubated for 5 mins in a water bath at 80°C and then centrifuged again and applied.

Milk

1. Centrifuge milk at 3500 g for 10 minutes.
2. Apply the supernatant directly to the microtiter plate pipetting below fat layer.

Honey

1. To 2 g honey add 4 mL double distilled water and vortex until dissolved.
2. Add 4 mL ethyl acetate and vortex 2mins and roll/shake for 10 minutes.
3. Centrifuge 10mins at 3000g room temperature
4. Transfer 1 mL of the upper ethyl acetate phase to a clean glass vial and evaporate the solvent at 50-70°C under a nitrogen or airstream to dryness.
5. Dissolve the dry residue with 500 µL diluted wash buffer by vortexing.
6. Apply sample to microtiter plate.

Assay Procedure

1. Prepare samples as described above.
2. Pipet:
   - 25 µL standards and prepared samples in duplicate into the appropriate wells
   - 100 µL Chloramphenicol-HRP conjugate into each well of the microtiter plate.
3. Cover the wells with the microtiter plate cover provided and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds or by tapping side of plate. Be careful not to spill contents.
4. Incubate in dark for 30 minutes at room temperature.
5. After incubation, remove the covering and vigorously shake the contents of these wells into a sink. Wash the strips using a wash bottle four times using the 1X wash buffer solution. Use 300 µL of washing buffer for each well and tap the plate for 10seconds for every wash. On the last wash the

remaining buffer in the wells should be removed by patting the plate dry on tissue paper/lint free paper

6. Pipet 125 µL of substrate solution into each well.
7. Allow the reaction to develop in the dark for 15 minutes incubate at room temperature.
8. Stop enzyme reaction by adding 100 µL of stop solution into each well. The blue colour will turn yellow upon addition.
9. After thorough mixing, measure absorbance at 450 nm (reference wavelength 630 nm if available), using an appropriate reader. A 4 parameter curve fit is recommended. The colour is stable for 30 minutes

Recommended working template

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1	Std 1	Smp 2	Smp 2								
B	Std 2	Std 2	Smp 3	Smp 3								
C	Std 3	Std 3										
D	Std 4	Std 4										
E	Std 5	Std 5										
F	Std 6	Std 6										
G	QC	QC										
H	Smp 1	Smp 1										

Std 0-Std 6: Standards
0; 0.05; 0.125; 0.5; 1; 4 ppb

Control

Samp1, Samp2, etc.: Samples

Calculation of results

1. Calculate the average optical density (OD 450 nm) for each set of reference standards or samples.
2. Construct a standard curve by plotting the mean optical density obtained for each reference standard against its concentration in ng/mL on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis.
3. Use the mean optical density value for each sample, determine the corresponding concentration of chloramphenicol in ng/mL from the standard curve. Depending on experience and/or the availability of software, other methods of data reduction may be employed. A 4 parameter curve fit is recommended.
4. The diluted samples must be further converted by the appropriate sample dilution factor. The factors are listed for each sample matrix in the sample preparation section.

Example of standard curve

The following table contains an example for a typical standard curve. The binding is calculated as percent of the absorption of the 0 ng/mL standard. These values are only an example and should not be used instead of the standard curve which has to be measured in each new test.

Chloramphenicol (ppb)	% binding B/B0
0	100
0.05	88
0.125	74
0.5	43
1	29
4	11

Results Evaluation

SHRIMP/PRAWN

The ELISA result should be multiplied by a factor of 0.25 to obtain the final Chloramphenicol concentration in the sample.

URINE

The ELISA result should be multiplied by a factor of 1 to obtain the final Chloramphenicol concentration in the sample.

MILK

The ELISA result should be multiplied by a factor of 1 to obtain the final Chloramphenicol concentration in the sample.

HONEY

The ELISA result should be multiplied by a factor of 1 to obtain the final Chloramphenicol concentration in the sample.