VERSION N13
CAT.NUMBER: B8048/B8096

BIO-SHIELD CHLORAMPHENICOL

ELISA TEST | In vitro analysis

for the quantitative determination of Chloramphenicol in serum/plasma, honey, eggs, milk, meat, muscle, seafood, and yogurt samples.

ProGnosis Biotech S.A. is ISO 9001:2015 certified by TÜV Hellas (TÜV NORD).

Use only the current version of Product Data Sheet enclosed with the kit.

Bio-Shield Chloramphenicol, B8048/B8096, is an immunoassay method that determines Chloramphenicol, in serum/plasma, honey, eggs, milk, muscle, seafood, and yogurt samples. The ELISA kit contains all reagents required for the immunoassay method. The ELISA test is adequate for 48/96 definitions (standards are included). A spectrophotometer for microtiter ELISA plate is required.

Matrices:
Serum/plasma, honey, eggs, milk, meat, muscle and seafood, and yogurt

- Sample preparation: Homogenization, extraction, centrifugation, evaporation and reconstitution
- Test time: (incubation time after samples and reagents preparation): 40 min
- Standard curve range: 0 - 2 ppb
- Shelf life: 12 months
- Storage: 2-8°C

This is an electronic version, please verify always the last one included in the kit.

Specifications

Matrix	Dilution factor	Range	LOD	LOQ	IC50 Range
Meat (beef, pork, poultry), muscle and seafood (e.g. fish, shrimp)	0.5	0 - 1ppb	0.005ppb	0.0125ppb	0.05 - 0.35ppb
Serum / Plasma	1	0 - 2ppb	0.01ppb	0.025ppb	0.1 - 0.7ppb
Honey	0.5	0 - 1ppb	0.005ppb	0.0125ppb	0.05 - 0.35ppb
Eggs	1	0 - 2ppb	0.01ppb	0.025ppb	0.1 - 0.7ppb
Milk	0.1	0 - 0.2ppb	0,001ppb	0,0025ppb	0.01 - 0.07ppb
Yogurt	0.5	0 - 1ppb	0.005ppb	0.0125ppb	0.05 - 0.35ppb

- The recovery of spiked extractions-matrices was 90.3% (CV = 8.1%).
- Each standards duplicates mean CV ≤ 6%.
- Cross Reactivity

X-reactive compound	Cross Reactivity (%)	X-reactive compound	Cross Reactivity (%)
Chloramphenicol	100	Nitrofurantoin, AHD, NP-AHD	<0.01
Dextramycin	<0.01	Furaltadone, AMOZ, NP-AMOZ	<0.01
Chloramphenicol base	<0.01	Furazolidone, AOZ, NP-AOZ	<0.01
Florfenicol	<0.01	Nitrofurazone, SEM, NP-SEM	<0.01
Thiamphenicol	<0.01	Chloramphenicol glucuronide	<0.01

1. Description

Bio-Shield Chloramphenicol is an ELISA test for the detection Chloramphenicol in serum/plasma, honey, eggs, milk, meat, muscle, seafood, and yogurt samples.

2. General Information

Chloramphenicol is a broad spectrum antibiotic against many bacteria in farm animals. Consumption of Chloramphenicol might be harmful for people since it is a known carcinogen and it might cause a broad range of disorders such as, bone marrow depression, aplastic anaemia and other blood disorders. For this reason, chloramphenicol is forbidden to be used in food production animals. Thus, accurate and rapid determination of Chloramphenicol presence in commodities is of paramount importance. The minimum reference point for action of Chloramphenicol according to Commision Regulation (EU) 2019/1871 is 0.15ppb.

3. Principle of the method

The quantitative test is based on the enzyme linked immunosorbent assay principles. The wells of the microtiter strips are coated with Chloramphenicol specific antibodies. Chloramphenicol is extracted from the samples with ethyl acetate. Chloramphenicol standards or samples and Chloramphenicol-HRP conjugate (detection solution) are added into the coated wells. Chloramphenicol-HRP conjugate binds to the binding sites of coated antibodies that are not already occupied by Chloramphenicol of standards or samples. Any unbound Chloramphenicol-HRP conjugate of detection solution is removed in a washing step. A chromogen substrate is added to the wells resulting in the progressive development of a blue colored complex with the detection antibody. The color development is then stopped by the addition of acid turning the resultant final product yellow. The measurement is made photometrically at 450 nm and the intensity of the produced colored complex is indirectly proportional to the concentration of Chloramphenicol present in the samples and standards.

4. Reagents Provided

Bio-Shield Chloramphenicol ELISA kit contains sufficient reagents and materials for 48/96 measurements (including standard tests).

Reagents (Store at 2-8ºC)	Quantity for 48 wells	Quantity for 96 wells	State	Vial cap color
Single-Break Strip Plate	48 wells	96 wells	Ready to use (precoated)	-
Dilution Microwells	48 wells	96 wells	Ready to use (green color)	-
Sealing film	2 sheets	2 sheets	Ready to use	-
Standards 1-5 (0, 0.025, 0.1, 0.5 and 2 ppb of Chloramphenicol in aqueous solution)	5 plastic vials (each 1.5 mL)	5 plastic vials (each 1.5 mL)	Ready to use	Brown
Standard Solution for Spiking 20 ppb of Chloramphenicol	1 plastic vial (3 mL)	1 plastic vial (3 mL)	Ready to use	Brown
Chloramphenicol detection solution	1 plastic vial (7.5mL)	1 plastic vial (15 mL)	Ready to use	Green
Wash Buffer	1 plastic vial (50 mL)	2 plastic vial (50 mL)	20X Concentrate (dilute in distilled water)	White
TMB Substrate	1 plastic vial (7,5mL)	1 plastic vial (15 mL)	Ready to use	Brown
Stop Solution	1 plastic vial (7,5mL)	1 plastic vial (15 mL)	Ready to use	White

5. Materials required but not provided

- A grinder sufficient to render sample to particle size of fine instant coffee
- Balance with 0 - 50 g measuring capability and Graduated cylinder - 100 mL
- Distilled or deionized water
- Ethyl acetate p.a. and n-Hexane ≥ 95%
- 20% Methanol and 10% Methanol
- Vortex mixer and/or blender and/or shaker
- Microtiter plate reader fitted with 450 nm filter
- Centrifuge, Waterbath and Timer
- Evaporator or Drying system with N_2 gas
- pH paper and sample tubes
- 100, 200 and 1000 µL adjustable single channel micropipettes with disposable tips (a repetitive pipette of 100 µL is acceptable for the steps of TMB and Stop Solution)
- 50 - 300 µL multi-channel micropipette with disposable tips and reservoirs

6. Storage Instructions

Store kit reagents between 2 and 8°C (35 - 46°F). Do not freeze any components provided. Reseal immediately the unused strips of the microtiter plate in the bag together with the desiccant bag provided and store at 2 - 8°C. After use remaining reagents should be returned to cold storage (2 - 8°C). Expiry of the kit and reagents is stated on the labels respectively and no quality guarantee is accepted after the expiration date. The expiry of the kit components can only be guaranteed if the components are stored properly as well as if the reagent is not contaminated by the first handling, in case of repeated use of one component. Because of the colorless TMB Substrate and standards light sensitivity, avoid the exposure to direct light. Do not interchange individual reagents between kits of different lot numbers.

7. Safety and Precautions for use

- Avoid any skin contact with Chloramphenicol standards, Stop Solution (8% H_3PO_4) and TMB (toxic). **Use gloves.** In case of contact, wash thoroughly with water.
- All reagents should be warmed in room temperature before use and covered when not in use. **Use a clean disposable plastic pipette tip for each reagent, in order to avoid cross contamination. When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.**
- Use a clean plastic container to prepare the wash buffer and all residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper into the well. Read the absorbance within 60 minutes after completion of the assay.

8. Indication of corruption of kit reagents

- The bluish coloration of the chromogen substrate before the ELISA test.
- A value of less than 0.7 absorbance units (ABS 450nm) for the Standard 1 (St1).

9. Sample and Reagents Preparation

9.1 Reagents preparation

Dilute the 20X solution concentrate 20 fold with distilled water to give a 1X working solution.

Preparation of Wash Buffer 1X: In case of the occurrence of crystals in the Wash Buffer, the warming by gentle dismantling (using hands) of the crystals is needed. Pour entire content of the solution concentrate (50ml) into a clean 1000ml graduated cylinder, rinse the vial with distilled or deionized water and pour the content again into the cylinder and fill to a final volume of 1000ml with distilled or deionized water. Mix gently to avoid foaming, transferring the final solution from cylinder to a clean bottle and back two times. The clean bottle with **1X Wash Buffer** working solution can be left out of the refrigerator during the method procedure and subsequent be stored 2 - 8°C for one month.

9.2 Meat (beef, pork, poultry), muscle and seafood (e.g. fish, shrimp)

A. Grind a representative sample (at least 5 g) until homogeneous.
B. Weight out a 3 g ground portion of the sample in a glass tube, add 6 mL ethyl acetate and vortex for 2 min (or roller for 10 min). **The ratio of sample to extraction solvent is 1:2 (w/v).**
C. Centrifuge 10 min at 3000g.
D. Transfer 4 mL of supernatant (ethyl acetate) into a new glass tube and evaporate solvent at 50°C to dryness using a slow air or nitrogen stream.
E. Reconstitute the dried residue in 1 mL n-hexane.
F. Add 1 mL of wash buffer solution 1X and vortex for 30 sec.
G. Centrifuge 10 min at 3000g.
H. Transfer the lower aqueous phase into a new tube. **The final dilution factor is 0.5.**
I. Use 150 µL directly in the immunoassay.

9.4 Serum / Plasma

A. Use 1 mL of serum/plasma, add 2 mL of ethyl acetate and vortex for 1 min.
B. Centrifuge 10 min at 3000g.
C. Transfer 1 mL of supernatant (ethyl acetate) into a new glass tube and evaporate solvent at 50°C to dryness using a slow air or nitrogen stream.
D. Reconstitute the dried residue in 0.5 mL wash buffer solution 1X. **The final dilution factor is 1.**
E. Use 150 µL directly in the immunoassay.

9.4 Honey

A. Weight out a 2 g portion of the sample in a glass tube, add 4 mL distilled water and mix to dissolve.
B. Add 4 mL ethyl acetate and vortex for 2 min (or roller for 10 min). **The ratio of sample to extraction solvent is 1:2 (w/v).**
C. Centrifuge 10 min at 3000g.
D. Transfer 1 mL of supernatant (ethyl acetate) into a new glass tube and evaporate solvent at 50°C to dryness using a slow air or nitrogen stream.
E. Reconstitute the dried residue in 0.5 mL wash buffer solution 1X and vortex for 30 sec.
F. Transfer the lower aqueous phase into a new tube. **The final dilution factor is 1.**
G. Use 150 µL directly in the immunoassay.

9.4 Eggs

A. Grind a representative sample (at least 5 g) until homogeneous.
B. Weight out a 2 g portion of the sample in a glass tube, add 8 mL ethyl acetate and vortex for 2 min (or roller for 10 min). **The ratio of sample to extraction solvent is 1:4 (w/v).**
C. Centrifuge 10 min at 3000g.
D. Transfer 4 mL of supernatant (ethyl acetate) into a new glass tube and evaporate solvent at 50°C to dryness using a slow air or nitrogen stream.
E. Reconstitute the dried residue in 1 mL n-hexane.
F. Add 1 mL of the wash buffer solution 1X and vortex for 30 sec.
G. Centrifuge 10 min at 3000g.
H. Transfer the lower aqueous phase into a new tube. **The final dilution factor is 1.**
I. Use 150 µL directly in the immunoassay.

9.4 Milk

A. Centrifuge 10 min at 3000g at 4°C.
B. Add 6 mL ethyl acetate in 3 mL of defatted milk and vortex for 2 min (or roller for 10 min). **The ratio of sample to extraction solvent is 1:2 (w/v).**
C. Centrifuge 5 min at 3000g.
D. Transfer 4 mL of supernatant (ethyl acetate) into a new glass tube and evaporate solvent at 50°C to dryness using a slow air or nitrogen stream.
E. Reconstitute the dried residue in 0.2 mL wash buffer solution 1X. **The final dilution factor is 0.1.**
F. Use 150 µL directly in the immunoassay.

9.5 Yogurt

A. Weight out a 4 g portion of the sample in a glass tube, add 6 mL ethyl acetate and vortex for 2 min (or roller for 10 min). **The ratio of sample to extraction solvent is 1:1,5 (w/v).**
B. Centrifuge 10 min at 3000g.
C. Transfer 3 mL of supernatant (ethyl acetate) into a new glass tube and evaporate solvent at 50°C to dryness using a slow air or nitrogen stream.
D. Reconstitute the dried residue in 1.5 mL n-hexane.
E. Add 1 mL of the wash buffer solution 1X and vortex for 30 sec.
F. Centrifuge 10 min at 3000g.
G. Transfer the lower aqueous phase into a new tube. **The final dilution factor is 0.5.**
H. Use 150 µL directly in the immunoassay.

NOTE 1: If an emulsion if formed in the upper layer, incubate the sample in a water bath (80°C) for 5 min. If necessary, centrifuge once more.
NOTE 2: Instead of n-hexane, iso-octane / chloroform (2/3 v/v) may be used. In this case use the upper phase for the analysis.

10. Method procedure

10.1 Assay Design: Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for standards. Considering that each sample and standard can be tested in single or in duplicate, create a layout. **NOTE:** Do not use more than 48 wells (six strips) in a single experiment.

CAUTION: Use the standards positions in duplicate as the **Example plate** layout below **NECESSARY**

	1	2	3	4	5	6	7	8	9	10	11	12
A	St1	St1										
B	St2	St2										
C	St3	St3										
D	St4	St4										
E	St5	St5										
F												
G												
H												

Example plate layout (example for a 5 point standard curve)

10.2 Bring all reagents to room temperature (19 - 24°C) before use. Remove the **standards** (Standard 1-5) and place one Dilution Microwells (green) in a microwell holder for each Standard and Sample to be tested in duplicate or single. Place **the appropriate number** of Antibody Coated Microtiter Wells in another microwell holder. Immediately reseal the unused strips of the microtiter plate in the bag together with the desiccant bag provided. The samples should be stored in *a cool place.*

10.3 Add **150µl** of **Chloramphenicol Detection Solution** to each Dilution Well. In case the experiment is run in single add **100µL** to each dilution well.

10.4 Using new pipette tip for each, add **150 µL** of each Standard **(Standard 1 - 5)** and prepared sample in duplicate (see Chapter 9) to appropriate Dilution Well containing the **Chloramphenicol Detection Solution**. Mix by priming pipetting at least 5 times. In case the experiment is run in single add **100µL** of each Standard and **100µL** of each prepared sample to each dilution well.

10.5 Using a multichannel pipette, transfer **100 µL** of contents from each Dilution Microwell to the corresponding Antibody Coated Microtiter Wells. Cover the microwells with the sealing film and incubate at room temperature for **20 min**.

10.6 Remove the sealing film and wash the plate as follows: Aspirate the liquid from each well into the sink and tap the holder of microwells upside down strongly (four times in a row) on an absorbent paper to insure the complete removal of liquid from the wells. Dispense **300 µL** of **Wash Buffer 1X** (see 9.1) into each well with wash bottle or multichannel micropipette using the proper reagent reservoir and shaking the plate manually for a few seconds. Repeat this process for another three times **(total 4 times). CAUTION:** It is important to not allow microwells to dry between working steps.

10.7 Aspirate the liquid as described above and add **100 µL** per well of **TMB Substrate** (pour 1ml per 8 wells in a reservoir). Cover the microwells with the sealing film, shaking the plate manually for a few seconds and incubate in the dark at room temperature for **20 min.**

10.8 Remove the sealing film and add **100 µL** per well of the **Stop Solution** to each well (pour 1ml per 8 wells in a reservoir). Mix gently by shaking again the plate manually.

10.9 **Measure the absorbance at 450 nm.** Read the absorbance value of each well (within 60 minutes after the step 10.8) on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm as the reference wave length (610 nm to 650 nm is acceptable).

11. Data Analysis

- **Automatically**

An assigned software, the Prognosis-Data-Reader, is available for free (contact: exports@prognosis-biotech.com) download in order to evaluate the Bio-Shield Chloramphenicol ELISA kit. The evaluation is carried out by a simple transfer of data values after the measurement.

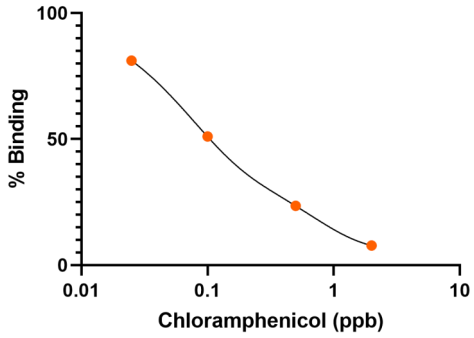
- **Manually**

Calculate the average absorbance values for each set of duplicate standards and samples. Ideally duplicates should be within 10% of the mean. Use the following calculation:

$$\frac{\text{Standard or sample absorbance}}{\text{Standard 1 absorbance}} \times 100 = \%\ \text{Binding}$$

The standard 1 is equal to 100 % and the absorbance values are quoted in percentages. The concentration of Chloramphenicol (ppb) in each sample is determined by extrapolating OD values against concentrations of Chloramphenicol in standard solutions using a two phase exponential decay standard curve with logarithmic X axis.

12. Example of Standard Curve (0 - 2 ppb)



13. Performance Evaluation

13.1 Reference Materials

Several reference materials are being used for the evaluation of each product of ProGnosis Biotech S.A. in the context of Quality Control performed by Quality Control Department. Please request a validation report, including the results, at exports@prognosis-biotech.com.

13.2 Proficiency Tests

All products participate frequently in Proficiency Tests. For more information, visit the individual product page in our website: **www.prognosis-biotech.com**

14. Method Summary

Total procedure time (after samples and reagents preparation): 40 min.

Mix 150 µL (or 100µL) of the Detection Solution with 150 µL (or 100µL) of the samples and standards in the Dilution Microwells

⇩

Transfer 100 µL from each well of the Dilution Microwells into the Antibody Coated Microwells

⇩

Incubate 20min at room temperature

⇩

Wash four times

⇩

Add 100 µl of TMB Substrate

⇩

Incubate 20min at room temperature

⇩

Add 100 µl Stop Solution

⇩

Read Absorbance at 450 nm within 60 min

B8048-B8096 Manual_Bio-Shield_Chloram_v13_en

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