

Chloramphenicol ELISA Kit

Enzyme-Linked Immunosorbent Assay for Detection of Chloramphenicol

Cat. No.: ELISA-CAP-001

96 TESTS

For *in vitro* use only

Please read this package insert completely before using this product

Background

Chloramphenicol (CAP) is a broad spectrum antibiotic which is repeatedly employed in animal production due to its significant pharmacokinetic and antibacterial properties. CAP is useful for the treatment of a number of bacterial infections including meningitis, plague, cholera and typhoid fever. Due to CAPs suspected carcinogenicity and also links with the development of aplastic anemia and bone marrow depression in humans, CAP has been banned for use in food-producing animals in the European Union (EU) and many other countries.

Intended use

The Biopanda CAP ELISA kit is a competitive immunoassay to quantitatively detect the presence of CAP in meat, seafood, milk, honey, egg (yolk) and dried milk samples.

Detection Limit – Meat / Seafood sample: 0.025 ppb

Milk sample: 0.05 ppb

Honey sample: 0.075 ppb

Egg (yolk) / Dried milk sample: 0.075 ppb

Recovery Rate – Meat sample: 75-125%

Seafood sample: 75-120%

Milk sample: 80-125%

Honey sample: 90-125%

Egg (yolk) / Dried milk sample: 80-120%

Specificity (Cross-Reactivity) - Chloramphenicol: 100%

Florfenicol: 0%

Principle

This test kit is based on a competitive enzyme-linked immunosorbent assay (ELISA) for the detection of CAP. An unknown amount of CAP present in the sample and the fixed amount of CAP antigens pre-coated onto the wells of microtiter plate/strips compete for the anti-CAP antibodies, which in turn are detected with enzyme conjugate. After incubation, the wells are washed and the bound enzyme is visualised by adding TMB solution. Any coloured product is measured at 450 nm after adding stop solution. The absorbance value of the developed colour is inversely proportional to the amount of CAP in the sample. The quantity of CAP in the test sample can be calculated using the standard curve constructed from the standards, and corrected for the sample dilution.

Storage and stability

- The kit should be stored at 2–8°C. Do not freeze.
- Unused test wells should be sealed and stored at 2–8°C.
- This kit is valid until the expiration date printed on the label.

Kit components supplied

Item #	Description	Quantity
1	Pre-coated microtitre plate	12 x 8 wells
2	CAP antibody solution	1 x 7 ml
3	Enzyme conjugate	1 x 7 ml
4	Wash buffer (20x concentrate)	1 x 30 ml
5	Assay diluent (5x concentrate)	1 x 20 ml
7	CAP standards (0, 0.05, 0.15, 0.45, 1.35, 4.05 ppb)	1 ml each
8	High concentrate of CAP standard (100 ppb)*	1 x 1 ml
9	TMB solution (ready to use)	1 x 12 ml
10	Stop solution (ready to use)	1 x 12 ml
11	Microplate sealer	1 piece
12	Package insert	1 copy

** This component is optional and only for the user to check the recovery rate of Chloramphenicol.*

Materials / Equipment required but not included with kit

- ELISA Microtiter plate reader equipped with 450/630 nm filters
- Single and multichannel micropipettes from 50 µl to 1.0 ml, with disposable pipette tips
- Plate shaking equipment
- Microplate washer or squeeze bottle
- Centrifuge
- Vortexer
- Centrifugal tubes
- Deionised water (ddH₂O)
- n-Hexane
- Ethyl acetate
- Sodium chloride

Precautions

- Please carefully read the instructions before use.
- Reagents should be brought to room temperature (RT, 18-25°C) prior to use.
- Do not use reagents after the expiration date. Do not use reagents from other kits with different Lot numbers.
- Avoid contact of skin and mucous membranes with reagents and sample extraction. If exposure should occur, immediately flush with water.
- Please wear protective gloves when using the kit. Consider all materials that are exposed to standards or samples to be contaminated.
- Use different tips when pipetting different reagents and samples.
- Keep the stop solution away from skin and eyes.

Preparation of Working Solutions

Wash buffer: Dilute the wash buffer concentrate (20x) 20-fold with deionized water (e.g. 10 ml wash buffer concentrate with 190 ml ddH₂O).

Assay diluent: Dilute the assay diluent concentrate (5x) 5-fold with deionized water (e.g. 10 ml assay diluent concentrate with 40 ml ddH₂O).

Note: Wash Buffer concentrate may form crystals at low temperature. Ensure that the crystals completely re-dissolve before dilution (by placing into a 37°C incubator or water bath if necessary).

Sample Preparation

Meat / Seafood (Dilution factor: 0.5)

- Weigh 3 g of homogenised sample into a 15 ml centrifugal tube, add 6 ml of ethyl acetate and mix thoroughly for 10 min.
- Centrifuge the sample at 4000 x g for 10 min at RT.
- Transfer 4 ml of supernatant to another tube, and dry with nitrogen gas at 50°C.
- Dissolve the dried residue in 1 ml of n-Hexane.
- Add 1 ml of diluted assay diluent and mix thoroughly for 2 min.
- Centrifuge at 4000 x g for 10 min at RT.
- Transfer 200 µl below the supernatant to an eppendorf tube for testing.

Milk (Dilution factor: 1)

- Measure 1 ml of milk sample into a 15 ml centrifugal tube, add 4ml of ethyl acetate and mix thoroughly for 10 minutes.
- Centrifuge the sample at 4000 x g for 10 min at RT.
- Transfer 2 ml of supernatant to another tube, and dry with nitrogen gas at 50°C.
- Dissolve the dried residue in 1 ml of n-Hexane.
- Add 500 µl of diluted assay diluent and mix thoroughly for 2 min.
- Centrifuge at 4000 x g for 10 min at RT.

Chloramphenicol ELISA Kit

Enzyme-Linked Immunosorbent Assay for Detection of Chloramphenicol

- Transfer 200 µl below the supernatant to an eppendorf tube for testing.

Honey (Dilution factor: 1.5)

- Weigh 1 g of honey sample into a 15 ml centrifugal tube and mix with 2 ml of saturated sodium chloride.
- Add 6 ml ethyl acetate and mix thoroughly for 10 min.
- Centrifuge the sample at 4000 × g for 10 min at RT.
- Transfer 3 ml of the supernatant to another tube, and dry with nitrogen gas at 50°C.
- Dissolve the dried residue in 750 µl of n-Hexane.
- Add 750 µl of diluted assay diluent and mix thoroughly for 2 min.
- Centrifuge at 4000 × g for 10 min at RT.
- Transfer 200 µl below the supernatant to an eppendorf tube for testing.

Egg (yolk) / Dried milk (Dilution factor: 1.5)

- Egg (yolk):** Weigh 1 g of homogenised egg yolk sample into a 15 ml centrifugal tube,
- Dried milk:** Weigh 1 g of dried milk into a 15 ml centrifugal tube, add 1 ml ddH₂O, and mix thoroughly.
- Add 6 ml ethyl acetate and mix thoroughly for 10 min.
- Centrifuge the sample at 4000 × g for 10 min at RT.
- Transfer 4 ml of the supernatant to another tube, and dry with nitrogen gas at 50°C.
- Dissolve the dried residue in 1 ml of n-Hexane.
- Add 1 ml of diluted assay diluent and mix thoroughly for 2 min.
- Centrifuge at 4000 × g for 10 min at RT.
- Transfer 200 µl below the supernatant to an eppendorf tube for testing.

NOTE: with some egg (yolk) samples, after drying there may be around 100µl of liquid fat residue left. This does not affect the results.

Test Procedure

- Ensure all reagents are equilibrated to RT prior to use. Swirl all reagents gently before use.
- Label each strip on its end tab to help identify them and should they become detached from the plate frame during the assay.
- To every well (except the two blank wells), add 50 µl of standard/sample to the wells in duplicate. Then add 50 µl of Enzyme conjugate solution to each well. Finally, add 50 µl of Antibody solution to each well.
- To the two blank wells, add 100 µl of diluted Assay diluent and 50 µl of Enzyme conjugate (No standards/samples and antibody solution).
- Cover the strips with plate sealer and shake gently to mix for 1 minute. Then incubate the plate for 30 minutes at 37°C in the dark.
- After incubation, remove the plate sealer and wash the strips 5 times with diluted Wash buffer, ensuring every well is filled. When washing is completed, tap the strips firmly on absorbent tissue to remove residual Wash buffer.

- Add 100 µl of the TMB solution to each well and incubate at 37°C for 10 minutes.
- Stop the reaction by adding 100 µl of Stop solution to each well in the same order as the TMB solution was added. Shake gently to mix.
- Measure absorbance at 450 nm (with 630nm as a reference filter) within 10 minutes of stopping.

Test validity

For the test to be valid, the mean absorbance of the zero standard (S1, 0 ppb) must be over 1.0.

Results calculation

The unknown CAP concentrations in the samples are determined from a standard curve. Calculate the mean absorbance value of the two blank wells and subtract that from the mean absorbance values of all the other wells.

Define the mean corrected absorbance value of the standards and samples as B. Define the mean corrected absorbance of the zero standard as B₀. The relative absorbance can therefore be calculated as:

$$\text{Relative absorbance (\%)} = \frac{B}{B_0} \times 100$$

Plot the relative absorbance of the standards against the standard concentration to obtain a standard curve. Using the relative absorbance value of a sample, the concentration can be found by interpolation. Remember to multiply by the dilution factor to obtain the true CAP concentration.

Interpolation can be performed by carrying out a 4-parameter logistic analysis, using a linear regression method, or point-to-point interpolation. Biopanda can provide an accompanying Excel spreadsheet calculator for this purpose.

Notes

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in this package insert and with adherence to good laboratory practice (GLP).
- Factors that might affect the performance of the assay include proper instrument function/calibration, cleanliness of glassware, quality of distilled or deionised water, accuracy of reagent and sample pipetting, washing technique, incubation time and temperature.

Thank you for purchasing Biopanda's CAP ELISA Test kit. Please read this manual carefully before operating to ensure proper use.

A recommended plate layout is shown below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	T2	T2	T10	T10	T18	T18	T26	T26	T34	T34	
B	S2	S2	T3	T3	T11	T11	T19	T19	T27	T27	T35	T35
C	S3	S3	T4	T4	T12	T12	T20	T20	T28	T28	T36	T36
D	S4	S4	T5	T5	T13	T13	T21	T21	T29	T29	T37	T37
E	S5	S5	T6	T6	T14	T14	T22	T22	T30	T30	T38	T38
F	S6	S6	T7	T7	T15	T15	T23	T23	T31	T31	T39	T39
G	B	B	T8	T8	T16	T16	T24	T24	T32	T32	T40	T40
H	T1	T1	T9	T9	T17	T17	T25	T25	T33	T33	T41	T41

'S' denotes the Standards in duplicate;

'B' denotes the Blank wells (see Step 4 of the Test Procedure);

'T' denotes the samples that are being tested in duplicate.

By following this recommended plate layout, the results from the microtiter plate reader can be copy & pasted directly into the accompanying spreadsheet calculator.

Revision date: 27/04/2021