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 an antibiotic useful for the treatment of a number of bacterial infections. This includes meningitis, plague, cholera, and typhoid fever. Common side effects include bone marrow suppression, nausea, and diarrhea. It belongs to amphenicol antibiotics, which has been banned in aquiculture. It is a broad spectrum fungicide, but the residues of CAP in aquatic products are very harmful to human health.

Intended use

The Biopanda CAP ELISA kit is a competitive immunoassay to quantitatively detect the presence of CAP in seafood and meat samples.

Detection Limit - Seafood/meat samples: 0.025ppb

Recovery Rate - Seafood/meat: 90-110%

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
1	1 × Pre-coated microtiter plate (12 × 8 microwells)
2	1 × CAP antibody solution (7 ml)
3	1 × Enzyme conjugate (7 ml)
4	1 × Wash buffer concentrate (20×, 30 ml)
5	1 × Assay diluent concentrate (3x, 30 ml)
6	6× CAP standards (0, 0.05, 0.15, 0.45, 1.35, 4.05 ppb, 1 ml each)
7	1 × High concentrate of CAP standard (100ppb, 1 ml) *
8	1 × TMB solution (12 ml)
9	1 × Stop solution (12 ml)
10	1 × Microplate sealer
11	1× Package Insert

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

Materials/equipment required but not included with kit

- ELISA Microtiter plate reader equipped with 450/630 nm filters
- Multichannel micropipette
- 20 µl, 50 µl, 100 µl, 200 µl,1000 µl micropipette tips
- Microplate washer or squeeze bottle
- Centrifuge
- Vortexer
- Centrifugal tubes
- Deionised water
- Ethyl Acetate
- n-Hexane

Precautions

- Please carefully read the instructions before use.
- Reagents should be brought to room temperature (RT, 20-25°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 H_2O).

Assay diluent: dilute the Assay diluent concentrate (3x) 3-fold with deionized water (e.g. 10 ml Assay diluent concentrate with 20 ml H_2O).

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

Seafood/Meat (Dilution factor: 0.5)

- Weigh out 3g of a homogenised sample into a 15 ml centrifugal tube and mix with 3 ml of extra pure water, then add 6ml ethyl acetate.
- Mix intensively for 10 min (head over head).
- Centrifuge the sample at 4000 ×*g* for 10 min at RT.
- Transfer 4ml of the supernatant to another tube, and dry with nitrogen gas at 60°C.
- Dissolve the dried residue in 2 ml of n-hexane.
- Add 1ml of diluted Assay Diluent and mix by vortexing at maximum speed for 2 min.
- Centrifuge at 4000 ×*g* for 10 min at RT. **Note: If an emulsion is present, put the tube in a water bath (80 °C) for approximately 5 minutes, or until the emulsion dissolves, and centrifuge again.**
- Take 500 µl of the lower aqueous phase and put into an Eppendorf tube for testing.

❖ ***The sample preparation method above is only a suggestion. The kit user can use their own methods to prepare samples.***

Test Procedure

1. Ensure all reagents are equilibrated to RT prior to use. Swirl all reagents gently before use.
2. Label each strip on its end tab to help identify them should they become detached from the plate frame during the assay.
3. 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.
4. To the two blank wells, add 100 µl of diluted Assay diluent and 50 µl of Enzyme conjugate (No standards/samples and antibody solution).
5. Cover the strips with the microplate sealer. Mix gently by shaking the plate for 1 minute, then incubate the plate for 30 minutes at 37°C in the dark.
6. 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.
7. Add 100 µl of the TMB solution to each well and incubate at 37°C for 10

minutes.

8. 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.
9. 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 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 B_0. The relative absorbance can therefore be calculated as:

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

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

1. 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).
2. 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.

A recommended plate layout is given as follows:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	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.

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