QUALICHEK™ Chloramphenicol ELISA

REF : KBFP1009

Ver 1.0

RUO

Quantitative testing of Chloramphenicol in samples, such as aquatic product, livestock, honey, milk and other crops/feed, etc.

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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Introduction:

Chloramphenicol (CAP) drugs, is used extensively in aquaculture due to its broad spectrum activity, and it is ready availability and low cost. CAP is a wide-spectrum antibiotic effective against many Gram-positive and Gram-negative bacteria, including several anaerobic organisms

Intended Use:

This kit is for quantitative testing for Chloramphenicol in sample, such as aquatic product, livestock, honey, milk and other crops/feed, etc.

Principle:

This kit uses Indirect-Competitive-ELISA as the method. It can detect CAP in samples, such as aquatic product, livestock, honey, milk and other crops/feed, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody, standard and other supplementary reagents. The microplate provided in this kit has been pre-coated with CAP. During the reaction, CAP in the samples or standard competes with CAP on the solid phase supporter for sites of CAP antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and TMB substrate is for color development. There is a negative correlation between the OD value of samples and the concentration of CAP. The concentration of CAP in the samples can be calculated by comparing the OD of the samples to the standard curve.

Materials Provided:

1. Coated Microtiter plate - 1x 96 wells
2. Standards Liquid -1mL each (0 ppb, 0.025 ppb, 0.075 ppb, 0.225 ppb, 0.675 ppb, 2.025 ppb)
3. High Concentrated Standard (100 ppb) - 1mL
4. HRP Conjugate - 11 mL
5. Antibody Working Solution - 5.5 mL
6. Substrate A - 6 mL
7. Substrate B - 6 mL
8. Stop Solution - 6 mL
9. Wash Buffer (20X) - 40 mL
10. Plate Sealer - 3 pieces

Material required but not provided:

Instrument: Microtiter plate reader, Printer, Homogenizer, Nitrogen Evaporators/Water bath, Oscillators, centrifuge, Graduated pipette, Balance (sensibility 0.01 g)
High-precision transferpettor:single channel (20-200 µL, 100-1000 µL), Multichannel (300 µL) micropipettes.
Reagents: Ethyl acetate, N-hexane, Acetonitrile, Natrium aceticum, Acetic acid, Potassium nitroprusside ($K_2Fe(CN)_5(NO)\cdot 2H_2O$), Glucuronidase, Zinc sulfate ($ZnSO_4\cdot 7H_2O$).

Storage Information:

1. Store main kit components at 2-8°C Avoid freeze / thaw cycles.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.

Sample pretreatment:

Bring all reagents and samples to room temperature before use.
Open the microplate reader in advance, preheat the instrument, and set the testing parameters.

1. Sample pretreatment Notice:

Experimental apparatus should be clean, and the pipette should be disposable to avoid cross-contamination during the experiment.

2. Solution preparation:

Solution 1: 0.36 M Potassium Nitroprusside Solution (milk, milk powder samples) : Dissolve 11.9 g of Potassium nitroprusside to 100mL of deionized water.
Solution 2: 1.04 M Zinc Sulfate Solution (milk, milk powder samples) Dissolve 29.8 g of Zinc sulfate to 100mL of deionized water.
Solution 3: 0.1 M, pH4.8 Natrium aceticum Buffer (urine sample): Dissolve 2.4 g of Natrium aceticum with 500 mL of deionized water, then add 1.2 mL of Acetic acid and mix fully.
Solution 4: Acetonitrile-water Solution Acetonitrile (V): Water (V) =84:16
Solution 5: Reconstitution Buffer (If sample is water sample, do not dilute it): Dilute the 2×Reconstitution Buffer with deionized water. (2×Reconstitution Buffer: deionized water=1:1) .The Reconstitution buffer can be store at 4℃ for a month
Solution 6: Wash Buffer: Dilute the 20×Concentrated Wash Buffer with deionized water. (20×Concentrated Wash Buffer (V): Deionized water (V) =1:19).

3. Sample pretreatment procedure:

Pretreatment of tissue, fish, shrimp, liver:
1. Weigh 3±0.05 g of homogenate sample into a 50 mL centrifuge tube, add 3 mL of deionized water and oscillate for 5 min, then add 6 mL of ethyl acetate and oscillate for 2 min. Centrifuge at 4000 rpm for 10min at room temperature.
2. Take 2 mL of the supernatant to another centrifuge tube, dry at 50-60℃ with Nitrogen Evaporators or water bath
3. Dissolve the residue with 1 mL of n-hexane, add 0.5 mL of Reconstitution buffer, and mix fully for 30 sec. Centrifuge at 4000 rpm for 5 min at room temperature.
4. Discard the upper organic phase, take 50 µL of the lower layer for analysis.

Note: Sample dilution factor: 0.5, minimum detection dose: 0.0125 ppb

Pretreatment of serum or plasma:
1. Take 1 mL of serum or plasma into centrifuge tube, add 2 mL of ethyl acetate and oscillate for 1 min, centrifuge at 4000 rpm for 5 min at room temperature.
2. Take the supernatant to another centrifuge tube, dry at 50-60℃ with Nitrogen Evaporators or water bath.
3. Dissolve the residue with 1 mL of n-hexane, added 1 mL of Reconstitution buffer, mix fully for 30 sec. Centrifuge at 4000 rpm for 5 min at room temperature.
4. Discard the upper organic phase, take 50 µL of the lower layer for analysis.

Note: Sample dilution factor: 1, minimum detection dose: 0.025 ppb

Pretreatment of urine:
1. Take 2 mL of urine into 50 mL centrifuge tube, mix with 0.5 mL of Natrium aceticum Buffer (0.1 M, pH4.8), then add 40 µL of glucuronidase, mix fully and hydrolysis at 37℃ for more than 2 hours (or overnight).
2. Bring the mixed solution in step 1 to room temperature, add 8 mL of ethyl acetate and oscillate for 1 min. Centrifuge at 4000 rpm for 10 min at room temperature.
3. Take 4 mL of the supernatant to another centrifuge tube, dry at 50-60℃ with Nitrogen Evaporators or water bath.
4. Dissolve the residue with 1 mL of Reconstitution buffer, mix fully.
5. Take 50 µL for detection and analysis.

Note: Sample dilution factor: 1, minimum detection dose: 0.025 ppb

Pretreatment of honey:

1. Weigh 2±0.05 g of honey into centrifuge tube, dissolved with 4 mL of deionized water, add 4 mL of ethyl acetate and oscillate for 2 min. Centrifuge at 4000 rpm for 10 min at room temperature.
2. Take 2 mL of supernatant to another centrifuge tube, dry at 50-60 ℃ with Nitrogen Evaporators or water bath.
3. Dissolve the residue with 0.5 mL of Reconstitution buffer, mix fully.
4. Take 50 µL for detection and analysis.

Note: Sample dilution factor: 0.5, minimum detection dose: 0.0125 ppb
(Minimum detection dose is 0.0125 ppb, quantitative lower limit is 0.05 ppb. As there are interference in some samples, 0.05 ppb is suggested as cut off value.)

Pretreatment of casing:
1. Wash and homogenize the casing, take 1±0.05 g of homogenate into 50 mL centrifuge tube, add 10 mL of ethyl acetate and oscillate for 2 min. Centrifuge at 4000 rpm for 10 min at room temperature.
2. Take 5 mL of the supernatant to another centrifuge tube, dry at 50-60℃ with Nitrogen Evaporators or water bath.
3. Dissolve the residue with 1 mL of n-hexane, add 0.5 mL of Reconstitution buffer, and mix fully for 30 sec. Centrifuge at 4000 rpm for 5 min at room temperature.
4. Discard the upper organic phase, take 50 µL of the lower layer for analysis.

Note: Sample dilution factor: 1, minimum detection dose: 0.025 ppb

Pretreatment of milk:
1. Centrifuge the milk at 4000 rpm for 10 min at 15℃, discard upper fat layer. Take 5 mL of fat free milk into 50 mL centrifuge tube, add 250 µL of Solution 1 and oscillate for 30 sec, then add 250 µL of Solution 2 and oscillate for 30 sec, centrifuge at 4000 rpm for 10 min at 15℃.
2. Take 2.2 mL of the supernatant to another centrifuge tube, add 4 mL of ethyl acetate and oscillate for 2 min, centrifuge at 4000 rpm for 10 min at room temperature.
3. Take 2 mL of supernatant to another centrifuge tube, dry at 50-60 ℃ with Nitrogen Evaporators or water bath.
4. Dissolved the residue with 0.5 mL of Reconstitution buffer, mix fully.
5. Take 50 µL for detection and analysis.

Note: Sample dilution factor: 0.5, minimum detection dose: 0.0125 ppb
(Minimum detection dose is 0.0125 ppb, quantitative lower limit is 0.025 ppb. As there are interference in some samples, 0.075 ppb is suggested as cut off value.)

Pretreatment of milk powder:
1. Weigh 2±0.05 g milk powder into centrifuge tube, dissolved with 10 mL deionized water, add 1 mL of Solution 1 and 1mL of Solution 2. Oscillate for 2 min and centrifuge at 4000 rpm for 10 min at 15℃.
2. Take 3.6 mL of the supernatant to another centrifuge tube, add 6 mL of ethyl acetate and oscillate for 5 min, centrifuge at 4000 rpm for 10 min at room temperature.
3. Take 4 mL of supernatant to another centrifuge tube, dry at 50-60 ℃ with Nitrogen Evaporators or water bath.
4. Dissolve the residue with 0.4 mL of Reconstitution buffer, mix fully.
5. Take 50 µL for detection and analysis.

Note: Sample dilution factor: 1, minimum detection dose: 0.025 ppb
(Minimum detection dose is 0.025 ppb, quantitative lower limit is 0.075 ppb. As there are interference in some samples, 0.075 ppb is suggested as cut off value.)

Pretreatment of eggs:
1. Weigh 3 ± 0.05 g of homogenate sample into 50 mL centrifuge tube, add 9 mL acetonitrile-water solution and oscillate for 2 min. Centrifuge at 4000 rpm for 10 min at 15℃.
2. Take 3 mL of the supernatant to another centrifuge tube, add 3 mL of deionized water and 4.5 mL of ethyl acetate. Oscillate for 1 min and centrifuge at 4000 rpm for 10 min at 15℃.
3. Take all the supernatant to another centrifuge tube, dry at 50-60 ℃ with Nitrogen Evaporators or water bath.
4. Dissolve the residue with 1 mL of n-hexane, add 2 mL of Reconstitution buffer, and oscillate for 30 sec. Centrifuge at 4000 rpm for 5 min at room temperature.
5. Discard the upper organic phase, take 50 µL of the lower layer for analysis.
   Note: Sample dilution factor: 2, minimum detection dose: 0.05 ppb (Minimum detection dose is 0.05 ppb, quantitative lower limit is 0.15 ppb.)

Pretreatment for feed:

1. Weigh 2±0.05 g of homogenate sample into 50 mL centrifuge tube, dissolved with 2 mL of deionized water, add 6 mL of ethyl acetate and oscillate for 2 min. Centrifuge at 4000 rpm for 10 min at 15℃.
2. Take 3 mL of the supernatant to another centrifuge tube, dry at 50-60℃ with Nitrogen Evaporators or water bath.
3. Dissolve the residue with 1 mL of n-hexane, added 1 mL of Reconstitution buffer, and oscillate for 30 sec Centrifuge at 4000 rpm for 5 min at room temperature.
4. Discard the upper organic phase, take 50 µL of the lower layer for analysis.

Note: Sample dilution factor: 1, minimum detection dose: 0.025 ppb

Pretreatment for water sample:

1. Take 0.5 mL of water sample into centrifuge tube, add 0.5 mL of 2×Reconstitution Buffer and oscillate for 1 min.
2. Take 50 µL for detection and analysis.

Note: Sample dilution factor: 2, minimum detection dose: 0.05 ppb

Assay procedure:

Centrifuge the sample again after thawing before the assay. Bring all reagents to room temperature for 30 min before use. **All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.**

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells.
2. **Add sample:** add 50 µL of Standard or Sample per well, then add 50 µL of antibody working solution, cover the plate with sealer, oscillate for 5 s gently to mix thoroughly, incubate for 30 min at 25℃.
3. **Wash:** uncover the sealer carefully; remove the liquid in each well. Immediately add 300 µL of wash buffer to each well and wash. Repeat wash procedure for 5 times, 30 s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **HRP Conjugate**: add 100µl of HRP conjugate to each well, incubate shading light for 30 min at 25^0C
5. **Wash**: repeat step 3.
6. **Color Development:** add 50 µL of Substrate Reagent A to each well, and then add 50 µL of Substrate Reagent B. Gently oscillate for 5 s to mix thoroughly. Incubate shading light for 15 min at 25℃ (The reaction time may be shortened or prolonged according to the depth of the color).
7. **Stop reaction:** add 50 µL of stop solution to each well, oscillate gently to mix thoroughly.
8. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm with a microplate reader (the 450/630 nm double wavelength is recommended). This step should be finished in 10 min after stop reaction.

Judgment of result:

Absorbance (%) =A/A0×100%

A: Average absorbance of standard or sample

A_0: Average absorbance of 0 ppb Standard

Drawing and calculation of standard curve

Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot. Add average absorbance value of sample to standard curve to get corresponding concentration. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.

For this kit, it is more convenient to use professional analysis software for accurate and fast analysis on a large number of samples

Notes:

1. The overall OD value will be lower when reagents have not been brought to room temperature before use or room temperature is below 25℃.
2. If the wells turn dry during the washing procedure, it will lead to bad linear standard curve and poor repeatability. Operate the next step immediately after wash.
3. Mix thoroughly and wash the plate completely. The consistency of wash procedure can strongly affect the reproducibility of this ELISA kit.
4. ELISA Microtiter plate should be covered by plate sealer. Avoid the reagents to strong light.
5. Do not use expired kit or reagents of different batches.
6. TMB should be abandoned if it turns color. When OD value of standard (concentration: 0)＜0.5 unit (A450nm＜0.5), it indicates the reagent may be deteriorated.
7. Stop solution is caustic; avoid contact with skin and eyes.

Technical specification:
Sensitivity: 0.025 ppb (ng/mL)
Reaction mode: 25℃, 30 min~ 30 min~15 min.
Detection limit: Tissue/liver/honey/milk--- 0.0125 ppb, Eggs/water sample---0.05 ppb.
Urine/serum/casing/feed/milk powder---0.025 ppb.
Reactivity: Chloramphenicol---100%, Thiamphenicol/Florfenicol＜0.1%.
Sample recovery rate: Tissue/ liver--- 85%±20%, Honey/ casing--- 85%±25%,
Milk/ feed---75%±25%, Urine/ serum---70%±20%,
Water sample---90%±20%.

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