CAP (Chloramphenicol) ELISA Kit

Catalog No: E-FS-E106

96T/96T*3

Version Number:	V1.2
Replace version:	V1.1
Revision Date:	2024.03.14

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

This kit uses Competitive-ELISA as the method for the quantitative detection. It can detect Chloramphenicol (CAP) in samples, such as, muscle, milk, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, standard and other supplementary reagents. The microtiter plate in this kit has been pre-coated with coupled antigen. During the reaction, CAP in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti-CAP antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each microtiter plate well, and substrate reagent is added 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.

Technical indicators

Reaction mode(Incubation time and temperature)**:** 25℃; 30 min, 15 min.

Detection limit: Honey, Finished milk, Milk powder, Yogurt---0.1 ppb; Feed---100ppb;
Muscle (pork, chicken, duck)--0.0125 ppb; Eggs, Serum----0.025 ppb;
Muscle (fish, shrimp, beef, mutton), Ham sausage, Raw milk ----0.05 ppb.

Cross-reactivity: Chloramphenicol---100%; Chloramphenicol Succinate--->100%;
Florfenicol---<0.1%; Chloramphenicol Base ---<5.0%

Sample recovery rate: 90%±30%;

Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1 mL each (ppb=ng/mL=ng/g) (0 ppb, 0.025 ppb, 0.1 ppb, 0.4 ppb, 1.6 ppb, 6.4 ppb)
20×Concentrated Wash Buffer	25 mL
10×Concentrated Sample Diluent	30 mL
5×Concentrated HRP Conjugate	2 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution

Other materials required but not supplied

Instrument: Microplate reader, Printer, Homogenizer, Nitrogen evaporators, Water bath, Vortex mixer Centrifuge, Graduated pipette, Balance (sensibility 0.01 g).

Micropipette: Single channel (20-200 μL, 100-1000 μL), Multichannel (30-300 μL).

Reagents: Ethyl acetate, N-hexane, $ZnSO4 \cdot 7H_2O$, $Na_2HPO_4 \cdot 12H_2O$, $NaH_2PO_4 \cdot 2H_2O$

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. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid t he kit to strong light.
5. **Each reagent is optimized for use in the E-FS-E106. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E106 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns color. When OD value of standard (concentration: 0) < 0.8 unit (A450nm < 0.8), it indicates the reagent may be deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.
8. As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.
9. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
10. **For mentioned sample fast and efficient extraction methods are included in the kit descriptio n. Please consult technical support for the applicability if other sample need to be tested.**
11. The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS, etc. can be used for quantitative confirmation.

Storage and expiry date

Store the kit at 2-8℃. Do not freeze any test kit components.

Return any unused microwells to their original foil bag and reseal them together with the desiccant provided and further store at 2-8℃.

Expiry date: expiration date is on the packing box.

Experimental preparation

Restore all reagents and samples to room temperature (25℃) 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

Please prepare solution according to the number of samples. Don't use up all components in the kit at once!

Solution 1: PB Solution (*for finished milk sample*)

Dissolve 67.84 g of **$Na_2HPO_4 \cdot 12H_2O$** and 1.7 g of **$NaH_2PO_4 \cdot 2H_2O$** to 1000 mL with deionized water. Mix fully.

Solution 2: 1 M $ZnSO_4$ Solution (*for raw milk, finished milk sample*)

Dissolve 28.8 g of **$ZnSO_4 \cdot 7H_2O$** to 100 mL with deionized water. Mix fully.

Solution 3: Sample Diluent

Dilute the **10×Concentrated Sample Diluent** with deionized water. (10×Concentrated Sample Diluent (V): deionized water (V) =1:9). Mix fully.

Solution 4: HRP Conjugate

Dilute the **5×Concentrated HRP Conjugate** with **Sample Diluent**. (5×Reconstitution Buffer (V): Sample Diluent (V) =1:4). Mix fully.

Solution 5: Wash Buffer

Dilute the **20×Concentrated Wash Buffer** with deionized water. (20×Concentrated Wash Buffer (V): Deionized water (V) =1:19). Mix fully.

3. Sample pretreatment

3.1 Pretreatment of honey sample:

(1) Weigh 3±0.05 g of honey into 50 mL centrifuge tube, add 6 mL of **Ethyl acetate** and vortex for 12 min to ensure mix fully. Centrifuge at 4000 r/min for 10 min at room temperature.

(2) Take 4 mL of supernatant to another centrifuge tube, dry at 50-60℃ with nitrogen evaporators or water bath. (Please do it in a ventilated environment.)

(3) Dissolve the residue with 1 mL of **Sample Diluent** (Solution 3), vortex for 30 s to mix fully.

(4) Take 50 μL for analysis.

Note: Sample dilution factor: 0.5, detection limit: 0.1 ppb

3.2 Pretreatment of muscle (fish, shrimp, livestock), ham sausage sample:

(1) Weigh 3±0.05 g of homogenate sample into a 50 mL centrifuge tube, add 6 mL of **Ethyl acetate** and vortex for 15 minutes to ensure mix fully. Centrifuge at 4000 r/min for 10 min at room temperature.

(2) Take 4 mL of supernatant to another centrifuge tube, dry at 50-60℃ with nitrogen evaporators or water bath.

(3) Dissolve the residue with 2 mL of **N-hexane**, add 1 mL of **Sample Diluent** (Solution 3), and vortex for 5 min. Centrifuge at 4000 r/min for 5 min at room temperature.

(4) Discard the upper organic phase and middle layer completely, take 50 μL of the lower water layer for analysis.

Note: Sample dilution factor: 0.5, detection limit: pork, chicken, duck ---0.0125 ppb
fish, shrimp, beef, mutton, ham sausage ----0.05 ppb

3.3 Pretreatment of raw milk (cattle, sheep) sample:

(1) Take 5 mL of raw milk into 50 mL centrifuge tube, add 5 mL of deionized water and vortex for 30 s, Centrifuge at 4000 r/min for 5 min.

(2) Take 6 mL of solution in middle layer to another centrifuge tube, add 0.6 mL of **1 M $ZnSO_4$ Solution** (Solution 2) and add 6 mL of **Ethyl acetate,** vortex for 2 min, and centrifuge at 4000 r/min for 5 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 1 mL of **Sample Diluent** (Solution 3) and 1 mL of **N-hexane**, and mix fully for 1 min. Centrifuge at 4000 r/min for 5 min at room temperature.

(5) Discard the upper organic phase and middle layer completely, take 50 μL of the lower water layer for analysis.

Note: Sample dilution factor: 1, detection limit: 0.05 ppb

3.4 Pretreatment of milk powder, yogurt sample:

(1) **Milk powder**: Weigh 0.5±0.05 g milk powder into 50 mL centrifuge tube, add 1 mL deionized water, Vortex for 2 min until completely dissolved.
Yogurt: Weigh 3±0.05 g homogenate sample into centrifuge tube.

(2) Add 6 mL of **Ethyl acetate** and vortex for 15 min to ensure mix fully. Centrifuge at 4000 r/min 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 2 mL of **N-hexane**, add 1 mL of **Sample Diluent** (Solution 3), and vortex for 5 min. Centrifuge at 4000 r/min for 5 min at room temperature.

(5) Discard the upper organic phase and middle layer completely, take 50 μL of the lower water layer for analysis.

Note: Sample dilution factor: yogurt: 0.5, milk powder: 3 detection limit: 0.1 ppb

3.5 Pretreatment of serum (swine) sample:

(1) Take 1 mL of fresh serum into 50 mL centrifuge tube, add 2 mL of **Ethyl acetate,** vortex for 2 min, and centrifuge at 4000 r/min for 5 min at room temperature.

(2) Take 1 mL of supernatant to another centrifuge tube, dry at 50-60℃ with nitrogen evaporators or water bath.

(3) Dissolved the residue with 500 μL of **Sample Diluent** (Solution 3), mix fully for 1 min.

(4) Take 50 μL for analysis.

Note: Sample dilution factor: 1, detection limit: 0.025 ppb

3.6 Pretreatment of feed sample:

(1) Weigh 1±0.05 g of homogenate sample into 50 mL centrifuge tube, add 10 mL of **Ethyl acetate** and vortex for 15 min to ensure full mixing. Centrifuge at 4000 r/min for 10 min at room temperature.

(2) Take 1 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 **Sample Diluent** (Solution 3), and vortex for 1min.

(4) Take 100 μL of solution to another centrifuge tube, add 900 μL of **Sample Diluent** (Solution 3), vortex for 30 s to mix fully.

(5) Take 50 μL for analysis.

Note: Sample dilution factor: 100, detection limit: 100 ppb

3.7 Pretreatment of eggs sample:

(1) Weigh 2±0.05 g of homogenate sample into 50 mL centrifuge tube, add 8 mL of **Ethyl acetate** and vortex for 2 min. Centrifuge at 4000 r/min for 10 min at room temperature.

(2) Take 4 mL of the supernatant to another centrifuge tube, dry at 50-60℃ with nitrogen evaporators or water bath.

(3) Dissolve the residue with 2 mL of **N-hexane**, add 1 mL of **Sample Diluent** (Solution 3), and vortex for 3 min.

Note: *If emulsification or uncleanness occurs, it should be demulsified in an 80℃ water bath or placed appropriately, and the next step should be carried out after the lower layer has clarified.*

(4) Centrifuge at 4000 r/min for 5 min at room temperature.

(5) Discard the upper organic phase and middle layer completely.

(6) After waiting for 25min, take 50 μL of the lower water layer for analysis.

(7) **Note: Sample dilution factor: 1, detection limit: 0.025 ppb**

3.8 Pretreatment of finished milk sample:

(1) Weigh 5±0.05 g of **finished milk** into centrifuge tube, add 5 mL of **PB Solution** (Solution 1) and vortex for 1 min. Centrifuge at 4000 r/min for 10 min at room temperature.

(2) Take 6 mL of solution in middle layer to another centrifuge tube (Avoid taking upper fat and lower solids), add 0.6 mL of 1 **M ZnSO4 Solution** (Solution 2) and 6 mL of **Ethyl ac etate**. Vortex for 1 min, centrifuge at 4000 r/min 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) Dissolve the residue with 1 mL of **Sample Diluent** (Solution 3) and 1 mL of **N-hexane**, mix fully for 1min. Centrifuge at 4000 r/min for 5 min at room temperature.

(5) Discard the upper organic phase and middle layer completely, take 50 μL of the lower water layer for analysis.

Note: Sample dilution factor: 1, detection limit: 0.1 ppb

Assay procedure

Restore all reagents and samples to room temperature (25℃) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8℃.

1. **Number:** Number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. **Standard and Samples need test in duplicate**
2. **Add Sample:** add 50 μL of **Standard** or **Sample** per well, then add 50 μL of **HRP Conjugate** (Solution 4), cover the plate with plate sealer. Oscillate for 10 s gently to mix thoroughly. Incubate at 25℃ for 30 min in shading light.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 260 μL of **Wash Buffer** (Solution 5) to each well and immerse for 30 s each time. Repeat wash procedure for 4 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. **Color Development:** add 50 μL of **Substrate Reagent A** to each well, and then add 50 μL of **Substrate Reagent B**. Gently oscillate for 10 s to mix thoroughly. Incubate at 25℃ in for 15 min shading light (The reaction time can be extended according to the actual color change).
5. **Stop Reaction:** add 50 μL of **Stop Solution** to each well, oscillate gently to mix thoroughly.
6. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm (reference wavelength 630 nm) with a microplate reader. This step should be finished in 5 min after stop reaction.

Result analysis

1. **Absorbance (%)=A/A_0×100%**

   A: Average absorbance of standard or sample

   A_0: Average absorbance of 0 ppb Standard

2. **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 form for accurate and fast analysis on a large number of samples.

Chloramphenicol (E-FS-E106) **Standard Curve**