CAP (Chloramphenicol) ELISA Kit

Catalog No: E-FS-E030

96T/96T*3

Version Number:	V1.2
Replace version:	V1.1
Revision Date:	2026.03.11

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, Enzyme Conjugate Concentrate, standard and other supplementary reagents.The CAP in the sample competes with the CAP enzyme complex for binding to the specific antibody of CAP that is immobilized in the enzyme-labeled plate wells. A color reaction is achieved through the catalytic action of the enzyme, 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, 30 min, 15 min.

Detection limit: Tissue, liver, honey, milk---0.0125 ppb; water sample---0.05 ppb; eggs---0.1 ppb; urine, serum, casing, feed, milk powder---0.025 ppb.

Cross-reactivity: Chloramphenicol ---100%; Thiamphenicol, Florfenicol--- <0.1%.

Sample recovery rate: Tissue, liver---85%±20%; honey, casing, eggs---85%±25%; milk, feed---75% ±25%; urine, serum---70%±20%; water sample---90%±20%;

Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1 mL each (ppb=ng/mL=ng/g) (0 ppb, 0.025 ppb, 0.075 ppb, 0.225 ppb, 0.675 ppb, 2.025 ppb)
HRP Conjugate	11 mL
Antibody Working Solution	5.5 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
20×Concentrated Wash Buffer	40 mL
2×Reconstitution Buffer	50 mL
Plate Sealer	1 piece
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, 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, NaOAc, Acetic Acid, $Na_2Fe(CN)_5(NO).2H_2O$, Glucuronidase, $ZnSO_4·7H_2O$.

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 th e kit to strong light.
5. **Each reagent is optimized for use in the E-FS-E030. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E030 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 time of each step of adding the sample solution, enzyme conjugate working solution, AB mixture and stop solution in plate well should not exceed 3 min.
12. 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℃. After opening, the kit is stable for up to 1 month.

Expiry date: expiration date is on the packing box.

Experimental preparation

Restore all reagents and samples to room temperature (25±2℃) 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: **0.36 M $Na_2Fe(CN)_5(NO).2H_2O$ Solution**

   Dissolve 10.7 g of $Na_2Fe(CN)_5(NO).2H_2O$ to 100 mL with deionized water, mix fully.

   Solution 2: **1.04 M $ZnSO_4·7H_2O$ Solution**

   Dissolve 29.8 g of **$ZnSO_4·7H_2O$** to 100 mL with deionized water, mix fully.

   Solution 3: **0.1 M pH4.8 NaOAc Solution**

   Dissolve 2.4 g of **NaOAc** to 500 mL with deionized water, add 1.2 mL of **Acetic Acid** mix fully.

   Solution 4: **Reconstitution Buffer**

   Dilute **2×Reconstitution Buffer** with deionized water (2×Reconstitution Buffer (V): Deionized water (V) =1: 1).

   Solution 5: **Wash Buffer**

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

3. **Sample pretreatment**

3.1 Pretreatment of tissue, fish, shrimp, liver sample:

(1) Remove fat from sample. Homogenize the representative sample with a homogenizer and mix fully.

(2) Weigh 3±0.05 g of homogenate edible sample into a 50 mL centrifuge tube, add 3 mL of deionized water, add 6 mL of **Ethyl acetate** and vortex for 2 min. Centrifuge at 4000 g for 10 min at room temperature.

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

(4) Dissolve the residue with 1 mL of **N-hexane**, add 0.5 mL of **Reconstitution Buffer** (Solution 4), vortex for 1 min. Centrifuge at 4000 g for 5 min at room temperature.

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

Note: Sample dilution factor: 0.5; detection limit: 0.0125 ppb

3.2 Pretreatment of serum sample:

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

(2) Take 1 mL of the upper phase to another centrifuge tube, dry at 50-60℃ with nitrogen evaporators.

(3) Dissolve the residue with 1 mL of **N-hexane**, add 0.5 mL of **Reconstitution Buffer** (Solution 4), vortex for 1 min. Centrifuge at 4000 g for 5 min at room temperature.

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

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

3.3 Pretreatment of urine sample:

(1) Take 2 mL of urine into the 15 mL centrifuge tube, add 0.5 mL of **0.1 M pH4.8 NaOAc Solution** (Solution 3), add 40 µL of **Glucuronidase**, mix well. Incubate at 37℃ for more than 2 hours (or overnight).

(2) After returning to room temperature, add 8 mL ethyl acetate and shake for 1 min. Centrifuge at 4000 g for 10 min at room temperature.

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

(4) Dissolve the residue with 1 mL of **Reconstitution Buffer** (Solution 4), and vortex for 30 s.

(5) Take 50 µL for analysis.

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

3.4 Pretreatment of honey sample:

(1) Weigh 2±0.05 g of honey sample into 50 mL centrifuge tube, add 4 mL of deionized water, add 4 mL of **Ethyl Acetate** and vortex for 2 min. Centrifuge at 4000 g for 10 min at room temperature.

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

(3) Dissolve the residue with 0.5 mL of **Reconstitution Buffer** (Solution 4), and vortex for 30 s.

(4) Take 50 µL for analysis.

Note: Sample dilution factor: 0.5 detection limit: 0.0125 ppb

3.5 Pretreatment of casing sample:

(1) The casings are cleaned and then homogenized, weigh 1±0.05 g of honey sample into 50 mL centrifuge tube, add 10 mL of **Ethyl Acetate** and vortex for 2 min. Centrifuge at 4000 g for 10 min at room temperature.

(2) Take 5 mL of supernatant to another centrifuge tube, dry at 50-60℃ with nitrogen evaporators.

(3) Dissolve the residue with 1 mL of **N-hexane**, add 0.5 mL of **Reconstitution Buffer** (Solution 4), vortex for 1 min. Centrifuge at 4000 g for 5 min at room temperature.

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

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

3.6 Pretreatment of raw milk (cattle) sample:

(1) Take 10 mL of milk into the 15 mL centrifuge tube, centrifuge at 4000 g for 10 min at 15℃.

(2) Discard the upper layer of fat and take 5 mL of the lower layer of skim milk into a new 50 mL centrifuge tube.

(3) Add 250 μL of **0.36 M $Na_2Fe(CN)_5(NO).2H_2O$ Solution** (Solution 1), Vortex for 30 s, then add 250 μL of **1.04 M $ZnSO_4·7H_2O$ Solution** (Solution 2), Vortex for 30 s, centrifuge at 4000 g for 10 min at 15℃.

(4) Take 2.2 mL of the supernatant to another 15 mL centrifuge tube, add 4 mL of **Ethyl acetate**, mix fully for 2 min, centrifuge at 4000 g for 10 min at room temperature.

(5) Take 2 mL of upper liquid (organic phase) to another 15 mL centrifuge tube, and dry at 50-60℃ with nitrogen evaporators or water bath. (Please do it in a ventilated environment.)

(6) Dissolve the residual with 0.5 mL of **Reconstitution Buffer** (Solution 4), oscillate until dissolved fully.

(7) Take 50 μL for analysis.

Note: Sample dilution factor: 0.5 detection limit: 0.0125 ppb

3.7 Pretreatment of milk powder sample:

(1) **Milk powder:** Weigh 2±0.05 g milk powder into 50 mL centrifuge tube, dissolved with 10 mL deionized water.

(2) Add 1 mL of **0.36 M $Na_2Fe(CN)_5(NO).2H_2O$ Solution** (Solution 1), then add 1 mL of **1.04 M $ZnSO_4·7H_2O$ Solution** (Solution 2), vortex for 30 s, centrifuge at 4000 g for 10 min at 15℃.

(3) Take 3.6 mL of supernatant to another centrifuge tube, add 6 mL of **Ethyl acetate**, mix fully for 5 min, centrifuge at 4000 g for 10 min at room temperature.

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

(5) Dissolve the residual with 0.4 mL of **Reconstitution Buffer** (Solution 4), oscillate until dissolved fully.

(6) Take 50 μL for analysis.

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

3.8 Pretreatment of eggs sample:

(1) Homogenize the sample use a homogenizer.

(2) Weigh 1±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 g for 5 min.

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

(4) Dissolve the residue with 2 mL of **N-hexane**, add 1 mL of **Reconstitution Buffer** (Solution 4), vortex for 2 min.

(5) Centrifuge at 4000 g for 5 min.

(6) Discard the upper organic phase, take 50 μL of the lower water layer for analysis.

Note: Sample dilution factor: 4 detection limit: 0.1 ppb

3.9 Pretreatment of feed sample:

(1) Homogenize the representative sample with a homogenizer and mix fully.

(2) Weigh 2±0.05 g of homogenate sample into 50 mL centrifuge tube, dissolved with 2 mL deionized water, then add 6 mL of **Ethyl acetate** and vortex for 2 min, centrifuge at 4000 g for 10 min at 15℃.

(3) Take 3 mL of the supernatant to another centrifuge tube, dry at 50-60℃ with nitrogen evaporators.

(4) Dissolve the residue with 1 mL of **N-hexane**, add 1 mL of **Reconstitution Buffer** (Solution 4), vortex for 1 min. Centrifuge at 4000 g for 5 min.

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

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

3.10 Pretreatment of water sample:

(1) Take 0.5 mL of clear water sample into the 1.5 mL centrifuge tube, add 0.5 mL of **2×Reconstitution Buffer**, vortex for 1 min.

(2) Take 50 µL for analysis.

Note: Sample dilution factor: 2 detection limit: 0.05 ppb

Assay procedure

Restore all reagents and samples to room temperature 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 **Antibody Working Solution**, 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 350 μL of **Wash Buffer** (Solution 5) to each well and immerse for 30 s each time. Repeat wash procedure for 5 times. 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 100 μL of **HRP Conjugate** to each well, cover the plate with plate sealer. Oscillate for 10 s gently to mix thoroughly. Incubate at 25℃ for 30 min in shading light.
5. **Wash:** repeat step 3.
6. **Color Development:** add 100 μL of **Substrate mixed solution** to each well (**Substrate Reagent A** and **Substrate Reagent B** are fully mixed at ratio 1:1 by volume, the mixture should be used within 5 min, avoid using metal containers or stirring the reagents), Gently oscillate for 10 s to mix thoroughly. Incubate at 25℃ for 15 min in shading light.
7. **Stop Reaction:** add 50 μL of **Stop Solution** to each well. Gently oscillate for 10 s to mix thoroughly.
8. **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 10 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-E030) **Standard Curve**