CAP (Chloramphenicol) ELISA Kit

Catalog No: E-FS-E113

96T/96T*3

Version Number:	V1.9
Replace version:	V1.8
Revision Date:	2025.08.14

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, antibody working solution, 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.

Limit of detection: Muscle, Liver, Honey ---0.0125 ppb; Eggs ---0.025 ppb; Water---0.05 ppb; Urine, Serum, Feed ---0.025ppb; Milk---0.0125 ppb; Milk powder---0.025 ppb; Cheese---0.125ppb.

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

Sample recovery rate: Muscle, Liver--- 90%±30%; Honey, Eggs --- 90%±30%; Water ---90%±20%; Milk, Feed, Milk powder ---75%±25%; Urine, Serum---70%±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)
Concentrated HRP Conjugate(100×)	60 µL
HRP Conjugate Diluent	6 mL
Antibody Working Solution	6 mL
Substrate Reagent	11 mL
Stop Solution	6 mL
10×Concentrated Wash Buffer	60 mL
10×Concentrated Sample Diluent	30 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, CH_3COONa, Acetic acid, $Na_2Fe(CN)_5(NO)\cdot 2H_2O$, β-glucuronidase (activity ≥ 1,000,000 units/g), $ZnSO_4$.

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-E113. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E113 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: 0.36 M $Na_2Fe(CN)_5(NO)\cdot 2H_2O$ Solution (*for milk, milk powder samples*)

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

   Solution 2: 1 M $ZnSO_4$ Solution (*for milk, milk powder samples*)

   Dissolve 16.15 g of **$ZnSO_4$** to 100 mL with deionized water, mix fully.

   Solution 3: 0.1 M, pH4.8 CH_3COONa Buffer *(for urine sample)*

   Dissolve 2.4 g of **CH_3COONa** with 500 mL of deionized water, then add 1.2 mL of **Acetic acid** and mix fully.

   Solution 4: Sample Diluent

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

   Solution 5: Wash Buffer

   Dilute the **10×Concentrated Wash Buffer** with deionized water. (10×Concentrated Wash Buffer (V): Deionized water (V) =1:9).

   Solution 6: HRP Conjugate

   Dilute the **Concentrated HRP Conjugate(100×)** with **HRP Conjugate Diluent** (**Concentrated HRP Conjugate(100×)** (V): **HRP Conjugate Diluent** (V) =1:99), mix fully.

3. **Sample pretreatment**

3.1 Pretreatment of muscle (fish, shrimp, livestock, poultry), 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 and mix fully, then add 6 mL of **Ethyl acetate** and vortex for 2 min. Centrifuge at 4000 r/min for 10 min at room temperature.

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

(4) Dissolve the residue with 1 mL of **N-hexane**, add 0.5 mL of **Sample Diluent** (Solution 4), and mix fully for 30 s. Centrifuge at 4000 r/min 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, Limit of detection: 0.0125 ppb

3.2 Pretreatment of serum (swine) sample:

(1) Take 1 mL of serum into centrifuge tube, add 2 mL of **Ethyl acetate** and vortex for 1 min, centrifuge at 4000 r/min for 5 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 **N-hexane**, add 0.5 mL of **Sample Diluent** (Solution 4), and mix fully for 30 s. Centrifuge at 4000 r/min 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, Limit of detection: 0.025 ppb

3.3 Pretreatment of urine (swine) sample:

(1) Take 2 mL of urine into centrifuge tube, mix with 0.5 mL of **0.1 M, pH4.8 CH_3COONa Buffer** (Solution 3), then add 40 μL of **β-glucuronidase**, mix fully and incubation at 37℃ for more than 2 hours (or overnight).

(2) Restore the mixed solution in step 1 to room temperature, add 8 mL of **Ethyl acetate** and vortex for 1 min. Centrifuge at 4000 r/min 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 **Sample Diluent** (Solution 4), mix fully.

(5) Take 50 μL for analysis.

Note: Sample dilution factor: 1, Limit of detection: 0.025 ppb

3.4 Pretreatment of honey sample:

(1) Weigh 3±0.05 g of honey sample into centrifuge tube, dissolved with 3 mL of deionized water, add 6 mL of **Ethyl acetate** and vortex for 2 min. Centrifuge at 4000 r/min 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 **Sample Diluent** (Solution 4), mix fully.

(4) Take 50 μL for analysis.

Note: Sample dilution factor: 0.5, Limit of detection: 0.0125 ppb

3.5 Pretreatment of milk sample:

(1) Centrifuge the milk at 4000 r/min for 10 min at 15℃, discard upper fat layer (**If a refrigerated centrifuge is not available, chill sample to approximate 15℃ prior to centrifugation.**). Take 5 mL of fat skim milk into 50 mL centrifuge tube, add 250 μL of **0.36 M $Na_2Fe(CN)_5(NO)\cdot 2H_2O$ Solution** (Solution 1) and vortex for 30 s, then add 250 μL of **1M $ZnSO_4$ Solution** (Solution 2) and vortex for 30 s, centrifuge at 4000 r/min for 10 min at 15℃.

(2) Take 2.2 mL of the supernatant to another centrifuge tube, add 4 mL of **Ethyl acetate** and vortex

for 2 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) Dissolved the residue with 0.5 mL of **Sample Diluent** (Solution 4), mix fully.

(5) Take 50 μL for analysis.

Note: Sample dilution factor: 0.5, Limit of detection: 0.0125 ppb

3.6 Pretreatment of milk powder sample:

(1) Weigh 2±0.05 g milk powder into 50 mL centrifuge tube, dissolved with 10 mL deionized water, add 1 mL of **0.36 M $Na_2Fe(CN)_5(NO)\cdot 2H_2O$ Solution** (Solution 1) and 1mL of **1.04 M $ZnSO_4$ Solution** (Solution 2) .Vortex for 2 min and centrifuge at 4000 r/min for 10 min at 15℃.

(2) Take 3.6 mL of the supernatant to another centrifuge tube, add 6 mL of **Ethyl acetate** and vortex for 5 min, 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 0.4 mL of **Sample Diluent** (Solution 4), mix fully.

(5) Take 50 μL for analysis.

Note: Sample dilution factor: 1, Limit of detection: 0.025 ppb

3.7 Pretreatment of Cheese powder sample:

(1) Homogenize the sample, use Homogenizer.

(2) Weigh 1±0.05 g sample into centrifuge tube, dissolved with 10 mL **Ethyl acetate**. Vortex for 2 min and centrifuge at 4000 r/min for 10 min.

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

(4) Dissolve the residue with 4 mL of **N-hexane,** mix fully. Add 1mL of **Sample Diluent (**Solution 4**)**. Vortex for 2 min and centrifuge at 4000 r/min for 10 min.

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

Note: Sample dilution factor: 5, Limit of detection: 0.125 ppb

3.8 Pretreatment of eggs sample:

(1) Homogenize the sample use a homogenizer. Weigh 2±0.05 g of homogenate sample into 50 mL centrifuge tube, add 0.6 mL **1.0 M $ZnSO_4$ Solution** (Solution 2) , then 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 1 mL of **N-hexane**, add 1 mL of **Sample Diluent** (Solution 4), and vortex for 30 s. Centrifuge at 4000 r/min 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, Limit of detection: 0.025 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 of deionized water, add 6 mL of **Ethyl acetate** and vortex for 2 min. Centrifuge at 4000 r/min for 10 min at 15℃.

(3) Take 3 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 **N-hexane**, add 1 mL of **Sample Diluent** (Solution 4), and vortex for 30 s. Centrifuge at 4000 r/min 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: 1, Limit of detection: 0.025 ppb

3.10 Pretreatment of water sample:

(1) Take 0.5 mL of clear water sample into 1.5 mL centrifuge tube (If the sample is cloudy, centrifuge at 4000 r/min for 10 minutes), add 0.5 mL of **2×Sample Diluent** and vortex for 1 min.

(2) Take 50 µL for analysis.

Note: Sample dilution factor: 2, Limit of detection: 0.05 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, 50 µL **HRP Conjugate** (Solution 6), 50 µL **Antibody Working Solution in sequently**, cover the plate with plate sealer. Oscillate for 5 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 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 100 µL of **Substrate Reagent** to each well. Gently oscillate for 10 s to mix thoroughly. Incubate at 25℃ in for 15 min shading light.
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 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 solution or sample

   A_0: Average absorbance of 0 ppb Standard solution

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 concentration on the x-axis, adopting four-parameter logic function to draw a plot. Add the average absorbance value 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.**

Chloramphenicol (E-FS-E113) **Standard Curve**