# CAP (Chloramphenicol) ELISA Kit

Catalog No: E-FS-E184

96T/96T*3

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|---|---|
| **Version Number:** | V1.2 |
| **Replace version:** | V1.1 |
| **Revision Date:** | 2025.12.17 |

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±2℃; 30 min, 15-20 min.

**Detection limit:** Honey, cured pork, sausage (beef, pork), milk powder, cold drinks, yogurt, finished milk---0.1 ppb; Ham sausage, raw chicken skin, steamed chicken skin, cartilage skewers, charcoal-grilled leg meat skewers, raw milk (bovine), raw milk (bovine, ovine)---0.05 ppb; Pork, chicken, duck---0.0125 ppb; Chicken liver---0.1 ppb; Fish, shrimp, crab meat, beef, mutton, pork liver, rabbit meat---0.05 ppb; Serum, eggs---0.025 ppb; Feed---100 ppb.

**Cross-reactivity:** Chloramphenicol ---100%; Chloramphenicol succinate---121%; Chloramphenicol base---< 5.0%; Methyl chloramphenicol, Florfenicol ---< 0.1%.

**Sample recovery rate:** 95%±25%.

## Kit components

| Item | Specifications |
|---|---|
| ELISA Microtiter plate | 96 wells |
| Standard Liquid | 1.5 mL each (ppb=ng/mL=ng/g)<br>(0 ppb, 0.025 ppb, 0.1 ppb, 0.4 ppb, 1.6 ppb, 6.4 ppb) |
| High-concentration Standard Liquid | 1 vial (10 ppb) |
| 5×Concentrated HRP Conjugate | 2 mL |
| Substrate Reagent A | 7 mL |
| Substrate Reagent B | 7 mL |
| Stop Solution | 7 mL |
| 20×Concentrated Wash Buffer | 25 mL |
| 10×Concentrated Sample Diluent | 30 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, $Na_2HPO_4 \cdot 12H_2O$, $NaH_2PO_4 \cdot 2H_2O$, $ZnSO_4 \cdot 7H_2O$.

## 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℃.
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-E184. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E184 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: **PB Buffer**

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

   Solution 2: **1 M $ZnSO_4$ Solution**

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

   Solution 3: **HRP Conjugate**

   Dilute **5×Concentrated HRP Conjugate** with deionized water. (5×Concentrated HRP Conjugate (V): Deionized water (V) =1:4).

   Solution 4: **Sample Dilution Buffer**

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

   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 muscle (pork, chicken, pork liver, chicken liver, fish, shrimp, crab meat, duck, beef, mutton, rabbit), cured pork, sausage (beef, pork), ham sausage 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 6 mL of **Ethyl acetate** and vortex for 1 min. Shake at 300 rpm for 10 minutes using an orbital shaker. Centrifuge at 4000 g for 10 min at room temperature.

(3) Take 4 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 2 mL of **N-hexane** and vortex for 10 s, add 1 mL of **Sample Dilution Buffer** (Solution 4), and mix fully with low-speed 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:** Sausage, cured meat (beef, pork), chicken liver---0.1 ppb; pork, chicken, duck ---0.0125 ppb; pork liver, fish, shrimp, crab meat, beef, lamb, rabbit, ham sample ---0.05 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, let stand for 5-10 minutes for phase separation.

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

(3) Dissolve the residue with 0.5 mL of **Sample Dilution Buffer (Solution 4)**, and mix fully for 30 s.

(4) Take 50 µL for analysis.

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

**3.3 Pretreatment of honey sample:**

(1) Weigh 3±0.05 g of honey sample into 50 mL centrifuge tube, dissolved with 6 mL of **Ethyl acetate** and vortex for 1 min. Shake at 300 rpm for 10 minutes using an orbital shaker. Centrifuge at 4000 g for 10 min at room temperature.

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

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

(4) Take 50 µL for analysis.

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

**3.4 Pretreatment of raw milk (bovine) sample:**

(1) Take 5 mL of the raw milk sample into centrifuge tube. Centrifuge at 5000 g for 15 min at 0-4℃, discard upper fat layer.

(2) Take 50 µL for analysis.

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

**3.5 Pretreatment of raw milk (bovine, ovine) sample:**

(1) Take 5 mL of the raw milk sample into 50 mL centrifuge tube. Dissolved with 5 mL deionized water and vortex for 30 s. Centrifuge at 4000 g for 5 min, take 6 mL of the middle layer into a new 50 mL centrifuge tube (avoiding taking the upper fat layer and the lower solid layer).

(2) Add 0.6 mL of **1 M $ZnSO_4$ Solution** (Solution 2) and 6 mL of **Ethyl acetate**, and mix fully for 30 s. Centrifuge at 4000 g for 5 min, take 2 mL of the supernatant into a new centrifuge tube.

(3) Dry at 50-60℃ with nitrogen evaporators. Add 1 mL of **Sample Dilution Buffer (Solution 4)** and 1 mL of **N-hexane** sequentially, and vortex for 30 s. Centrifuge at 4000 g for 5 min, completely discard the upper n-hexane layer and the intermediate impurity layer.

(4) Take 50 µL for analysis.

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

**3.6 Pretreatment of milk powder, cold drinks, yogurt (method 1) sample:**

(1) **Milk powder:** Weigh 0.5 g milk powder into 50 mL centrifuge tube, dissolved with 1 mL deionized water, and vortex until the powder dissolves. (then proceed to step 3)

(2) **Cold drinks, yogurt:** Weigh 3±0.03 g of the homogenized sample into 50 mL centrifuge tube. (then proceed to step 3)

(3) Add 6 mL of **Ethyl acetate**, vortex vigorously for 2 min and shake at 300 rpm on an orbital shaker for 10 min. Centrifuge at 4000 g for 10 min.

(4) Take 4 mL of supernatant to another centrifuge tube, dry at 50-60℃ with nitrogen evaporators.
**Note: If the sample volume of milk powder is less than 4 mL, it is recommended to take 3 mL for nitrogen blowdown (dilution factor: 4).**

(5) Dissolve the residue with 2 mL of **N-hexane**, then add **Sample Dilution Buffer (Solution 4)**, vortex for 30 s. Centrifuge at 4000 g for 5 min, completely discard the upper n-hexane layer and the intermediate impurity layer.

(6) Take 50 µL for analysis.
**Note: Sample dilution factor:** milk powder: 3, cold drinks, yogurt: 0.5
**detection limit: 0.1 ppb**

**3.7 Pretreatment of eggs sample:**

(1) Homogenize the sample use a homogenizer.

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

(3) Take 4 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 **Sample Dilution Buffer (Solution 4)**, and high-speed vortex for 1 min.
**Note: If emulsification or turbidity occurs, it should be demulsified in a water bath at 80°C or left to stand appropriately. and then proceed to the next step after the lower layer becomes clear.**

(5) Centrifuge at 4000 g for 5 min. Discard the upper organic phase and the intermediate impurity layer, and let it stand for 20 - 25 minutes until the n-hexane has completely volatilized.

(6) Take 50 µL for analysis.
**Note: Sample dilution factor: 1 detection limit: 0.025 ppb**

**3.8 Pretreatment of feed sample:**

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

(2) Weigh 1±0.05 g of homogenate sample into 50 mL centrifuge tube, dissolved with 10 mL of **Ethyl acetate** and vortex for 1 min, and shake at 300 rpm on an orbital shaker for 10 min. Centrifuge at 4000 g for 10 min.

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

(4) Dissolve the residue with 1 mL **Sample Dilution Buffer (Solution 4)**, and vortex for 1 min. Take 100 μL of the mixture to another centrifuge tube, add 900 μL **Sample Dilution Buffer (Solution 4),** and vortex for 30 s.

(5) Take 50 μL for analysis.

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

**3.9 Pretreatment of raw chicken skin, steamed chicken skin, cartilage skewers, charcoal-grilled leg meat skewers 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 8 mL of **Ethyl acetate** and vortex for 1 min, and shake at 300 rpm on an orbital shaker for 10 min. Centrifuge at 4000 g for 10 min.

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

(4) Dissolve the residue with 3 mL of N-hexane and vortex for 10 s, add 1 mL of **Sample Dilution Buffer (Solution 4)**, and low-speed vortex for 1 min. Centrifuge at 4000 g for 5 min. Discard the upper organic phase and the intermediate impurity layer completely.

(5) Take 50 μL for analysis.

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

**3.10 Pretreatment of finished milk, yoghurt (method 2) sample:**

(1) Weigh 5±0.05 g of processed milk sample into 50 mL centrifuge tube. Add 5 mL **PB Buffer (Solution 1)** ， and vortex for 30 s. Centrifuge at 4000 g for 10 min.

(2) Take 6 mL of the intermediate aqueous phase to another 50 mL centrifuge tube.

**Note: Avoid taking the upper fat and lower solid**

(3) Add 0.6 mL **1 M ZnSO4 Solution (Solution 2)** and 6 mL of **Ethyl acetate**, and vortex for 1 min. Centrifuge at 4000 g for 10 min, take 2 mL of the upper ethyl acetate phase into a new centrifuge tube. Dry at 50-60℃ with nitrogen evaporators.

(4) Add 1 mL of **Sample Dilution Buffer (Solution 4)**, then add 1 mL of **N-hexane**, vortex for 30 s. Centrifuge at 4000 g for 5 min, completely discard the upper n-hexane layer and the intermediate impurity layer.

(5) Take 50 μL for analysis.

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

## Assay procedure

Restore all reagents and samples to room temperature (25±2℃) 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 **HRP Conjugate** (Solution 3), cover the plate with plate sealer. Oscillate for 10 s gently to mix thoroughly. Incubate at 25±2℃ 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 15-30 s each time. Repeat wash procedure for 4 times, 15-30 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 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±2℃ for 15-20 min in shading light.
5. **Stop Reaction:** add 50 μL of **Stop Solution** to each well. Gently oscillate for 10 s 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-E184) **Standard Curve**