Elabscience®

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CAP (Chloramphenicol) ELISA Kit

Catalog No: E-FS-E030

96T

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.

Phone: 240-252-7368(USA) 240-252-7376(USA)
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. It can detect Chloramphenicol (CAP) in samples, such as tissue, milk, honey and feed, 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 TMB substrate 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

Sensitivity: 0.05 ppb (ng/mL)

Reaction mode:25℃, 30 min~ 30 min~15 min.

Detection limit: Tissue, Liver, Honey, Milk--- 0.025 ppb; Eggs, Water ---0.1 ppb;
Urine, Feed, Milk powder ---0.05 ppb.

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

Sample recovery rate: Tissue, Lliver--- 85%±20%; Honey --- 85%±25%; Water---90%±20%.
Milk, Feed, Milk powder, Eggs ---75%±25%; Urine ---70%±20%.

Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1mL each (0 ppb, 0.05 ppb, 0.15 ppb, 0.45 ppb, 1.35 ppb, 4.05 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	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, Oscillators, Centrifuge, Graduated pipette, Balance (sensibility 0.01 g).

High-precision transferpettor: Single channel (20-200μL, 100-1000 μL), Multichannel (300 μL).

Reagents: Ethyl acetate, N-hexane, Acetonitrile, Sodium acetate trihydrate (CH_3COONa), Acetic acid, Sodium nitroferricyanide dihydrate ($Na_2Fe(CN)_5NO \cdot 2H_2O$), β-glucuronidase (activity ≥ 1,000,000 units/g), Zinc sulfate heptahydrate ($ZnSO4 \cdot 7H_2O$).

Experimental preparation

Restore 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 Sodium nitroferricyanide dihydrate Solution (for milk and milk powder sample)

   Dissolve 10.7 g **$Na_2Fe(CN)_5NO \cdot 2H_2O$** to 100mL with deionized water.

   Solution 2: 1.04 M ZnSO4 Solution (for milk and milk powder sample)

   Dissolve 29.8 g **$ZnSO4 \cdot 7H_2O$** to 100mL with deionized water.

   Solution 3: 0.1 M, pH4.8 Sodium acetate Buffer

   Dissolve 2.4 g **CH_3COONa** to 500 mL with deionized water, then add 1.2 mL of **Acetic acid**, 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 2×reconstitution buffer and use it directly).

   Dilute the **2×Reconstitution Buffer** with deionized water. (2×Reconstitution Buffer (V): Deionized water (V) =1:1).The Reconstitution buffer can be store at 4℃ for a month.

   Solution 6: 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, livestock), liver sample:

(1) Weigh 3±0.05 g of homogenate into 50 mL centrifuge tube, add 3 mL of deionized water and mix fully, then add 6 mL of **Ethyl acetate** and oscillate for 2 min. Centrifuge at 4000 r/min for 10min at room temperature.

(2) Remove 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** (Solution 5), and mix fully by shaking for 30s. Centrifuge at 4000 r/min for 5 min at room temperature.

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

Note: Sample dilution factor: 0.5, minimum detection limit: 0.025 ppb

3.2 Pretreatment of urine (swine) sample:

(1) Take 2 mL of urine into 50 mL centrifuge tube, add 0.5 mL of **0.1 M, pH4.8 Sodium acetate** (Solution 3) with mix fully, then add 40 μL of **β-glucuronidase**, mix fully and hydrolysis at 37℃ above 2 hours (or overnight).

(2) Restore the solution to room temperature, add 8 mL of **Ethyl acetate** and oscillate for 1 min. Centrifuge at 4000 r/min for 10 min at room temperature.

(3) Remove 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** (Solution 5), mix fully.

(5) Take 50 μL for analysis.

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

3.3 Pretreatment of honey sample:

(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 r/min for 10 min at room temperature.

(2) Remove 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** (Solution 5), mix fully.

(4) Take 50 μL for analysis.

Note: Sample dilution factor: 0.5, minimum detection limit: 0.025 ppb

(Minimum detection limit is 0.025 ppb, as there are interference in some samples, 0.1 ppb is suggested as cut off value.)

3.4 Pretreatment of milk sample:

(1) Centrifuge milk at 4000 r/min for 10 min at 15℃, discard upper layer fat. Take 5 mL of skim milk into 50 mL centrifuge tube, add 250 μL of **0.36 M Sodium nitroferricyanide dihydrate Solution** (Solution 1) and oscillate for 30s, then add 250 μL of **1.04 M $ZnSO_4$ Solution** (Solution 2) and oscillate for 30s, centrifuge at 4000 r/min for 10 min at 15℃.

(2) Removed 2.2 mL of the supernatant to another centrifuge tube, add 4 mL of **Ethyl acetate** and oscillate for 2 min, centrifuge at 4000 r/min for 10 min at room temperature.

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

(4) Dissolved the residue with 0.5 mL of **Reconstitution Buffer** (Solution 5), mix fully.

(5) Take 50 μL for detection and analysis.

Note: Sample dilution factor: 0.5, minimum detection limit: 0.025 ppb

(Minimum detection limit is 0.025 ppb, as there are interference in some samples, 0.15 ppb is suggested as cut off value.)

3.5 Pretreatment of milk powder sample:

(1) Weigh 2±0.05 g milk powder into centrifuge tube, dissolved with 10 mL deionized water, add 1 mL of **0.36 M Sodium nitroferricyanide dihydrate Solution** (Solution 1) and 1mL of **1.04 M $ZnSO_4$ Solution** (Solution 2). Oscillate well and centrifuge at 4000 r/min for 15 min at 15℃.

(2) Removed 3.6 mL of the supernatant to another centrifuge tube, add 6 mL of **Ethyl acetate** and oscillate for 5 min, centrifuge at 4000 r/min for 10 min at room temperature.

(3) Remove 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** (Solution 5), mix fully.

(5) Take 50 μL for analysis.

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

(Minimum detection limit is 0.05 ppb, as there are interference in some samples, 0.15 ppb is suggested as cut off value.)

3.6 Pretreatment of eggs sample:

(1) Weigh 3±0.05 g of homogenate into 50 mL centrifuge tube, add 9 mL **Acetonitrile-water Solution** (Solution 4) and oscillate for 2 min. Centrifuge at 4000 r/min for 10 min at 15℃.

(2) Remove 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 r/min for 10 min at 15℃.

(3) Remove 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** (Solution 5), and mix fully by shaking for 30s. Centrifuge at 4000 r/min for 5 min at room temperature.

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

Note: Sample dilution factor: 2, minimum detection limit: 0.1 ppb

(Minimum detection limit is 0.1 ppb, as there are interference in some samples, 0.3 ppb is suggested as cut off value.)

3.7 Pretreatment of feed sample:

(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 r/min 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** (Solution 5), and oscillate for 30s. 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, minimum detection limit: 0.05 ppb

3.8 Pretreatment of 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 limit: 0.1 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 **Antibody Working Solution**, cover the plate with plate sealer. Oscillate for 5s gently to mix thoroughly. Incubate at 25℃ for 30 min with shading light.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 300 μL of **Wash Buffer** (Solution 6) to each well and wash. Repeat wash procedure for 5 times, 30s intervals/time. Invert the plate and pat it against thick clean with 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. Incubate at 25℃ for 30 min with shading light.
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 5s to mix thoroughly. Incubate at 25℃ for 15 min with shading light. (The reaction time can be extended according to the actual color change).
7. **Stop reaction:** add 50 μL of **Stop Solution** to each well, gently oscillate for 5s 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 (%)=$A/A_0 \times 100\%$**

   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.

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 kit to strong light.
5. **Do not use expired kit, reagents of different batches and reagents that do not belong to this kit**.
6. TMB (Substrate Reagent A or Substrate Reagent B) should be abandoned if it turns blue color. When OD value of standard (concentration: 0)＜0.5 unit (A450nm＜0.5), it indicates the reagent 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. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
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 valid period

Store at 2~8℃ for 1 year. Avoid freeze.

Please store the opened kit at 2~8℃, protect from light and moisture. The valid period is 1 months.

Expiry date: expiration date is on the packing box.