Elabscience®

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CAP (Chloramphenicol) ELISA Kit

Catalog No: E-FS-E044

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 Indirect-Competitive-ELISA as the method. It can detect Chloramphenicol (CAP) in samples, such as aquatic, livestock, honey, milk and other crops/feed, etc. This kit is composed of Micro ELISA Plate, HRP conjugate, antibody, standard and other supplementary reagents. The micro-plate provided in this kit has been pre-coated with CAP. During the reaction, CAP in the samples or standard competes with CAP on the solid phase supporter for sites of CAP antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and TMB substrate is 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: 0.025 ppb (ng/mL)

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

Detection limit: Tissue/liver/honey/milk--- 0.0125 ppb, Eggs/water sample---0.05 ppb.
Urine/serum/casing/feed/mike powder---0.025 ppb.

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

Sample recovery rate: Tissue/ liver--- 85%±20%, Honey/ casing--- 85%±25%,
Milk/ feed---75%±25%, Urine/ serum---70%±20%，
Water sample---90%±20%.

Kit components

Item	Specifications
Micro ELISA Plate	96 wells
Standard Liquid (black cap)	1mL each (0 ppb, 0.025 ppb, 0.075 ppb, 0.225 ppb, 0.675 ppb, 2.025 ppb)
High Concentration Standard (100 ppb)	1 mL
HRP Conjugate (red cap)	11 mL
Antibody Working Solution (blue cap)	5.5 mL
Substrate Reagent A (white cap)	6 mL
Substrate Reagent B (black cap)	6 mL
Stop Solution (yellow cap)	6 mL
20×Concentrated Wash Buffer (white cap)	40 mL
2×Reconstitution Buffer (yellow cap)	50 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Other supplies required

Instrument: Microplate reader, Printer, Homogenizer, 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, Acetic acid, Potassium nitroprusside ($K_2Fe(CN)_5(NO)\cdot 2H_2O$), Glucuronidase, Zinc sulfate ($ZnSO_4\cdot 7H_2O$).

Experimental preparation

Bring all reagents and samples to room temperature before use.

Open the micro-plate 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 Potassium Nitroprusside Solution (milk, milk powder)

Dissolve 11.9 g of Potassium nitroprusside to 100mL of deionized water.

Solution 2: 1.04 M Zinc Sulfate Solution (milk, milk powder)

Dissolve 29.8 g of Zinc sulfate to 100mL of deionized water.

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

Add 2.4 g of Zinc sulfate to 500 mL of 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 aquiform, do not dilute it)

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

Solution 6: Wash Buffer

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

3. Sample pretreatment

3.1 Pretreatment of tissue, fish, shrimp, liver:

(1) Weigh 3±0.05 g of homogenate into 50 mL EP tube, add 3 mL of deionized water and oscillate for 5 min, then add 6 mL of ethyl acetate and oscillate for 5 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 or air.

(3) Dissolve the residue with 1 mL of n-hexane, add 0.5 mL of Reconstitution buffer, and mix fully by shaking for 30 sec. Centrifuge at 4000 r/min for 5 min at room temperature.

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

Note: Sample dilution factor: 0.5, minimum detection dose: 0.0125 ppb

3.2 Pretreatment of serum, plasma:

(1) Take 1 mL of serum or plasma into EP tube, add 2 mL of ethyl acetate and oscillate for 1 min, centrifuge at 4000 r/min for 5 min at room temperature.

(2) Remove the supernatant to another centrifuge tube, dry at 50-60℃ with nitrogen or air.

(3) Dissolve the residue with 1 mL of n-hexane, added 1 mL of Reconstitution buffer, mix fully by for shaking 30 sec. Centrifuge at 4000 r/min for 5 min at room temperature.

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

Note: Sample dilution factor: 1, minimum detection dose: 0.025 ppb

3.3 Pretreatment of urine:

(1) Take 2 mL of urine into 50 mL EP tube, mix with 0.5 mL of sodium acetate buffer (0.1 M, pH4.8), then add 40 μL of glucuronidase, mix fully and hydrolysis at 37℃ above 2 hours (or overnight).

(2) Take 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 or air.

(4) Dissolve the residue with 1 mL of Reconstitution buffer, mix fully.

(5) Take 50 μL for detection and analysis.

Note: Sample dilution factor: 1, minimum detection dose: 0.025 ppb

3.4 Pretreatment of honey:

(1) Weigh 2±0.05 g of honey into EP tube, dissolved with 4 mL of deionized water, add 4 mL of ethyl acetate and oscillate for 1 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 or air.

(3) Dissolve the residue with 0.5 mL of Reconstitution buffer, mix fully.

(4) Take 50 μL for detection and analysis.

Note: Sample dilution factor: 0.5, minimum detection dose: 0.0125 ppb

(Minimum detection dose is 0.0125 ppb, quantitative lower limit is 0.05 ppb. As there are interference in some samples, 0.05 ppb is suggested as cut off value.)

3.5 Pretreatment of casing:

(1) Wash and homogenize the casing, take 1±0.05 g of homogenate into 50 mL EP tube, add 10 mL of ethyl acetate and oscillate for 2 min. Centrifuge at 4000 r/min for 10 min at room temperature.

(2) Remove 5 mL of the supernatant to another centrifuge tube, dry at 50-60℃ with nitrogen or air.

(3) Dissolve the residue with 1 mL of n-hexane, add 0.5 mL of Reconstitution buffer, and mix fully by shaking for 30 sec. Centrifuge at 4000 r/min for 5 min at room temperature.

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

Note: Sample dilution factor: 1, minimum detection dose: 0.025 ppb

3.6 Pretreatment of milk:

(1) Centrifuge milk at 4000 r/min for 10 min at 15℃, discard upper layer fat. Take 5 mL of fat free milk into 50 mL EP tube, add 250 μL of Potassium nitroprusside solution (Solution 1) and oscillate for 30 sec, then add 250 μL of Zinc sulfate solution (Solution 2) and oscillate for 30 sec, centrifuge at 4000 r/min for 10 min at room temperature.

(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 air.

(4) Dissolved the residue with 0.5 mL of Reconstitution buffer, mix fully.

(5) Take 50 μL for detection and analysis.

Note: Sample dilution factor: 0.5, minimum detection dose: 0.0125 ppb

(Minimum detection dose is 0.0125 ppb, quantitative lower limit is 0.025 ppb. As there are interference in some samples, 0.075 ppb is suggested as cut off value.)

3.7 Pretreatment of milk powder:

(1) Weigh 2±0.05 g milk powder into EP tube, dissolved with 10 mL deionized water, add 1 mL of Potassium nitroprusside solution and 1mL of Zinc sulfate solution. Oscillate well and centrifuge at 4000 r/min for 15 min at room temperature.

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

(3) Remove4mL of supernatant to another centrifuge tube, dry at 50-60℃ with nitrogen or air.

(4) Dissolve the residue with 0.4 mL of Reconstitution buffer, mix fully.

(5) Take 50 μL for detection and analysis.

Note: Sample dilution factor: 1, minimum detection dose: 0.025 ppb

(Minimum detection dose is 0.025 ppb, quantitative lower limit is 0.075 ppb. As there are interference in some samples, 0.075 ppb is suggested as cut off value.)

3.8 Pretreatment of eggs:

(1) Weigh 3±0.05 g of homogenate into 50 mL EP tube, add 9 mL acetonitrile-water solution and oscillate for 2 min. Centrifuge at 4000 r/min for 15 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 or air.

(4) Dissolve the residue with 1 mL of n-hexane, add 2 mL of Reconstitution buffer, and mix fully by shaking for 30 sec. Centrifuge at 4000 r/min for 5 min at room temperature.

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

Note: Sample dilution factor: 2, minimum detection dose: 0.05 ppb

(Minimum detection dose is 0.05 ppb, quantitative lower limit is 0.15 ppb.)

3.9 Pretreatment for fodder:

(1) Weigh 2±0.05 g of crushed homogenate into 50 mL EP 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) Remove 3 mL of the supernatant to another centrifuge tube, dry at 50-60℃ with nitrogen or air.

(3) Dissolve the residue with 1 mL of n-hexane, added 1 mL of Reconstitution buffer, and mix fully by shaking for 30 sec. Centrifuge at 4000 r/min for 5 min at room temperature.

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

Note: Sample dilution factor: 2, minimum detection dose: 0.05 ppb

3.10 Pretreatment for water sample:

(1) Take 0.5 mL of water sample into EP tube, add 0.5 mL of Reconstitution buffer and oscillate for 1 min.

(2) Take 50 μL for detection and analysis.

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

Assay procedure

Centrifuge the sample again after thawing before the assay. Bring all reagents to room temperature before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Number:** Number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells.
2. **Add sample:** Add 50 μL of Standard or Sample per well, then add 50 μL antibody working solution, cover the plate with sealer. Oscillate for 5 sec gently to mix thoroughly. Incubate for 30 min at 25℃in the dark.
3. **Wash:** Uncover the sealer carefully, remove the liquid in each well. Immediately add 250 μL of wash buffer to each well and wash. Repeat wash procedure for 5 times, 30 sec 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. **HRP Conjugate:** Add 100 μL of HRP conjugate to each well. Incubate for 30 min at 25℃ in the dark.
5. **Wash:** Repeat Step 3.
6. **Color Development:** Add 50 μL of substrate solution A to each well, and then add 50 μL of substrate solution B. Gently oscillate for 5 sec to mix thoroughly. Incubate for 15 min at 25℃ in the dark.
7. **Stop reaction:** Add 50 μL of stop solution to each well, gently oscillate for 5 sec.
8. **OD Measurement:** Determine the optical density (OD value) of each well at 450 nm with a micro-plate reader (the 450/630 nm double wavelength is recommended). 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 software for accurate and fast analysis on a large number of samples.

Notes

1. Overall OD value will be lower when reagents is not brought to room temperature before use or room temperature is below 25℃.
2. During the washing procedure, if the wells turn dry, it will lead to bad linear standard curve and poor repeatability, move on to the next step immediately after wash.
3. Mix thoroughly and wash the plate completely. The consistency of wash procedure can strongly affect the repro ducibility of this ELISA kit.
4. Micro ELISA plate should be covered by plate sealer. Avoid the reagents to strong light.
5. Do not use expired kit and reagents of different batches.
6. TMB should be abandoned if it turns color. When OD value of standard (concentration: 0)＜0.5 unit($A_{450\ nm}$＜0.5), it indicates reagent is deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.

Storage and valid period

Storage: Store at 2-8℃. Avoid freeze / thaw cycles.

Valid Period: 1 year, production date is on the packing box.