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 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 canbe calculated by comparing the OD of the samples to the standard curve.

Technical indicators

Sensitivity:0.05ppb (ng/mL)

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

Detection limit: Tissue/liver/honey/milk--- 0.025ppb, Eggs/water sample---0.1ppb.
Urine/serum/casing/feed/mike powder---0.05ppb.

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

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

Kit components

Item	Specifications
Micro ELISA Plate	96 wells
Standard Solution	1mL each (0ppb,0.05ppb,0.15ppb,0.45ppb,1.35ppb,4.05ppb)
High Concentration Standard(100ppb)	1mL
HRPConjugate(red cap)	11mL
Antibody Working Solution(blue cap)	5.5mL
Substrate Reagent A(white cap)	6mL
Substrate Reagent B(black cap)	6mL
Stop Solution(yellow cap)	6mL
20×Concentrated Wash Buffer(white cap)	40mL
2×Redissolved Buffer(yellow cap)	50mL
Product Description	1 copy

Other supplies required

Instrument: Microplatereader, Printer, Homogenizer, Oscillators, Centrifuge, Graduated pipette, Balance (sensibility 0.01g).

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

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: 0.36M Potassium nitroprussidesolution (milk, milk powder)

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

Solution2: 1.04M Zinc sulfate solution (milk, milk powder)

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

Solution3:0.1M, pH4.8Sodium acetate buffer (urine)

Add 2.4g Zinc sulfate to 500mL deionized water, then add 1.2mLAcetic acid, mix fully.

Solution 4: Acetonitrile-water solution

Acetonitrile (V): Water (V) =84:16

Solution 5: Reconstitution fluid (If sample is aquiform, do not dilute it). For sample re-dissolution. That is, 2×Re-dissolve solution: deionized water(volume)=1:1. Store at 4℃, valid for 1 month.

3. **Sample pretreatment**

3.1 Pretreatment of tissue, fish, shrimp, liver:

(1) Weigh 3±0.05gof homogenate into 50mL EP tube, add 3mLdeionized water and oscillate for 5 min, then add 6mL ethyl acetate and oscillate for 5min. Centrifuge at 4000r/min for 10min at room temperature.

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

(3) Dissolvetheresiduewith1mLofn-hexane, added0.5mL Re-dissolve solution, mix fully by shakingfor 30 seconds. Centrifuge at 4000r/min for 5min at room temperature.

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

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

3.2 Pretreatment of serum, plasma:

(1) Take1mL of serum or plasma into EP tube, add 2mL of ethyl acetate and oscillate for 1min, centrifuge at 4000r/min for 5min at room temperature.

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

(3) Dissolvetheresiduewith1mLofn-hexane,added1mLRe-dissolvesolution,mix fully by for shaking 30seconds.Centrifuge at 4000r/min for 5min at room temperature.

(4) Remove the hexane upper layer, take 50μL of the lower layer for analysis.
Note: Sample dilution factor: 1, minimum detection dose: 0.05 ppb

3.3 Pretreatment of urine:

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

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

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

(4) Dissolvetheresiduewith1mLRe-dissolve solution, mix fully.

(5) Take 50μL for detection and analysis.
Note:Sample dilution factor: 1, minimum detection dose: 0.05 ppb

3.4 Pretreatment of honey:

(1) Weigh2±0.05ghoney into EP tube, dissolved with 4mL deionized water, add 4mL ethyl acetate and oscillate for 1min. Centrifuge at 4000r/min for 10min at room temperature.

(2) Remove2mL of supernatant to another centrifuge tube, dry with nitrogen evaporators at 50-60℃.

(3) Dissolvetheresiduewith0.5mLRe-dissolve solution, mix fully.

(4) Take 50μLfor detection and analysis.
Note: Sample dilution factor: 0.5, minimum detection dose: 0.025 ppb
(Minimum detection dose is 0.025ppb, quantitative lower limit is 0.1ppb. As there are interference in some samples, 0.1ppb is suggested as cut off value.)

3.5 Pretreatment of casing:

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

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

(3) Dissolvetheresiduewith1mLofn-hexane, add0.5mLRe-dissolve solution, and mix fully byshakingfor 30seconds. Centrifuge at 4000r/min for 5min at room temperature.

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

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

3.6 Pretreatment of milk:

(1) Centrifuge milk at 4000r/min for 10min at 15℃, discard upper layer fat. Take 5mL of fat free milk into 50mL EP tube, add 250μLPotassium nitroprusside solution(Solution1) and oscillate for 30s, then add 250μL Zinc sulfate solution(Solution 2) and oscillate for 30s, centrifuge at 4000r/min for 10min at room temperature.

(2) Removed 2.2mL of the supernatant to another centrifuge tube, add4mL ethyl acetate and oscillate for 2min, centrifuge at 4000r/min for 10min at room temperature.

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

(4) Dissolvedtheresiduewith0.5mLRe-dissolve solution, mix fully.

(5) Take 50μLfor detection and analysis.

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

(Minimum detection dose is 0.025ppb, quantitative lower limit is 0.05ppb. As there are interference in some samples, 0.15ppb is suggested as cut off value.)

3.7 Pretreatment ofmilk powder:

(1) Weigh2±0.05gmilk powder into EP tube, dissolved with 10mL deionized water, add 1mLPotassium nitroprusside solution(Solution1) and 1mL Zinc sulfate solution(Solution2). Oscillate well andcentrifuge at 4000r/min for 15min at room temperature.

(2) Removed 3.6mL of the supernatant to another centrifuge tube, add6mL ethyl acetate and oscillate for 2min, centrifuge at 4000r/min for 10min at room temperature.

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

(4) Dissolvetheresiduewith0.4mLRe-dissolve solution, mix fully.

(5) Take 50μLfor detection and analysis.

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

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

3.8 Pretreatment of eggs:

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

(2) Remove 3mL of the supernatant to another centrifuge tube, add 3mL deionized water and 4.5mL ethyl acetate. Oscillate for 1minand centrifuge at 4000r/min for 10min at 15℃.

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

(4) Dissolvetheresiduewith1mLofn-hexane, add2mLRe-dissolve solution, and mix fully by shakingfor 30seconds. Centrifuge at 4000r/min for 5min at room temperature.

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

Note: Sample dilution factor: 2, minimum detection dose: 01ppb

(Minimum detection dose is 0.1ppb, quantitative lower limit is 0.3ppb.)

3.9 Pretreatment for fodder:

(1) Weigh2±0.05gof crushed homogenate into 50mL EP tube, dissolved with 2mLdeionized water, add 6mL ethyl acetate and oscillate for 2min. Centrifuge at 4000r/min for 10min at 15℃.

(2) Remove3mL of the supernatant to another centrifuge tube, dry with nitrogen evaporators at 50-60℃.

(3) Dissolvetheresiduewith1mLofn-hexane, added1mLRe-dissolve solution, and mix fully by shakingfor 30seconds. Centrifuge at 4000r/min for 5min at room temperature.

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

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

3.10 Pretreatment for water sample:

(1) Take 0.5 mL of water sample into EP tube, add0.5mL2×Re-dissolve solution and oscillate for 1min.

(2) Take 50μLfor 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.**Dilute 40mL of concentrated wash buffer into 800mLwash working buffer with deionized or distilled water.

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 50μL of Standard, Blank, or Sample per well, then add 50μL antibody working solution, cover the plate with sealer. Oscillate for 5s gently to mix thoroughly. Incubate for 30min at 25℃in the dark.
3. **Wash:** Uncover the sealer carefully, remove the liquid in each well. Immediately add 250μL of wash working buffer to each well and wash. Repeat wash procedure for 5 times, 30s 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 100μL HRP conjugate to each well. Incubate for 30min at 25℃ in the dark.
5. **Wash:**Repeat step 3.
6. **Color Development:** Add 50μL of substrate solution A to each well, and then add 50μL of substrate solution B. Gently oscillate for 5s to mix thoroughly. Incubate for 15min at 25℃in the dark.
7. **Stopreaction:**Add 50μLof stop solution to each well, gently oscillate for 5s.

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 10min 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 valueof 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 below25℃.
(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_{450nm}＜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.