Technical Manual

CAP (Chloramphenicol) ELISA Kit

- **Catalogue Code: FSES0031**
- **Competitive ELISA Kit**
- **Research Use Only**

Contents

1. Key features and Sample Types

Sensitivity:

0.025 ppb (ng/mL)

Assay Procedure:

25°C, 30 min~ 30 min~15 min.

Detection Limit:

Muscle, Liver - 0.0125 ppb; Honey, Water - 0.05 ppb; Eggs - 0.1 ppb Urine, Serum, Feed - 0.025 ppb; Milk, Milk powder - 0.075 ppb

Cross Reactivity:

Chloramphenicol - 100%; Thiamphenicol, Florfenicol < 0.1%.

Sample Recovery rate:

Muscle, Liver - 85%±20%; Honey - 85%±25%; Water - 90%±20%; Milk, Feed, Milk powder, Eggs - 75%±25%; Urine, Serum - 70%±20%.

Storage:

2-8°C for 6 months.

Expiry:

See Kit Label

2. Storage

Store the kit at 2~8°C. 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°C.

3. Test Principle

This kit uses a Competitive-ELISA method. 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.

4. Kit Contents

Each kit contains reagents for 96 assays including:

No.	Component	96-WellKit
1	ELISA Microtiter plate	96 wells
2	Standards	1mL each (0 ppb, 0.025 ppb, 0.075 ppb, 0.225 ppb, 0.675 ppb, 2.025 ppb)
3	HRP Conjugate	11 mL
4	Antibody Working Solution	5.5 mL
5	Substrate Reagent A	6 mL
6	Substrate Reagent B	6 mL
7	Stop Solution	6 mL
8	20×Concentrated Wash Buffer	40 mL
9	2×Reconstitution Buffer	50 mL
10	Plate Sealer	3 pieces
11	Sealed Bag	1 piece
12	Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Additional materials required:

Other materials required but not supplied

- **Instrument:** Microplate reader, Printer, Homogenizer, Nitrogen evaporators, Water bath, Vortex mixer, Centrifuge, Graduated pipette, Balance (sensitivity 0.01 g).
- **Micropipette:** Single channel (20-200 μL, 100-1000 μL), Multichannel (30-300 μL).
- **Reagents:** Ethyl acetate, N-hexane, Acetonitrile, CH_3COONa, Acetic acid, $Na_2Fe(CN)_5(NO)\cdot 2H_2O$, β-glucuronidase (activity≥1,000,000 units/g), $ZnSO4\cdot 7H_2O$.

5. Experimental Preparation

Bring all reagents and samples to room temperature (25°C) before use.

Open the microplate reader in advance, preheat the instrument, and set the testing parameters.

1. Sample pre-treatment 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 100mL with deionized water, mix fully.

Solution 2: 1.04 M $ZnSO_4$ Solution (*for milk, milk powder samples*)
Dissolve 29.8 g of **ZnSO4·7H $_2$O** to 100mL with deionized water.

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: Reconstitution Buffer
(for fish, shrimp, livestock, liver, serum, urine, honey, milk, milk powder, eggs, feed sample)
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°C for a month.

Solution 5: Wash Buffer
Dilute the **20×Concentrated Wash Buffer** with deionized water. (20×Concentrated Wash Buffer (V): Deionized water (V) =1:19).

3. Sample pre-treatment

Targets may be distributed unevenly, resulting in no detection. To avoid this, ensure to take sufficient samples when sampling.

3.1 Pre-treatment of muscle (fish, shrimp, livestock), liver sample:

1. Remove the skin, bone of sample. Homogenize the sample use Homogenizer.
2. Weigh 3±0.05 g of homogenate edible sample into a 50 mL centrifuge tube, add 3 mL of deionized water and vortex and mix, then add 6 mL of **Ethyl acetate** and vortex for 2 min. Centrifuge at 4000 r/min for 10min at room temperature.
3. Take 2 mL of the supernatant to another centrifuge tube, dry at 50-60°C with nitrogen evaporators or water bath.
4. Dissolve the residue with 1 mL of **N-hexane**, add 0.5 mL of **Reconstitution Buffer** (Solution 4), and mix fully for 30s. 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, detection limit: 0.0125 ppb

3.2 Pre-treatment of serum (swine)* sample:

(*Data validated in swine serum but pre-treatment can be applied for serum samples of multiple species.)

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 the supernatant to another centrifuge tube, dry at 50-60°C with nitrogen evaporators or water bath.
3. Dissolve the residue with 1 mL of **N-hexane**, added 0.5 mL of **Reconstitution Buffer (**Solution 4**)**, and mix fully 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, detection limit: 0.025 ppb

3.3 Pre-treatment of urine (swine) sample:

(*Data validated in swine urine but pre-treatment can be applied for urine samples of multiple species.)

1. Take 2 mL of urine into 50 mL centrifuge tube, mix with 0.5 mL of **CH_3COONa Buffer** (Solution 3) **(0.1 M, pH4.8)**, then add 40 µL of **β-glucuronidase**, mix fully and incubation at 37°C 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°C with nitrogen evaporators or water bath.
4. Dissolve the residue with 1 mL of **Reconstitution Buffer (**Solution 4**)**, mix fully.
5. Take 50 µL for analysis.
   Note: Sample dilution factor: 1, detection limit: 0.025 ppb

3.4 Pre-treatment 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 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°C with nitrogen evaporators or water bath.
3. Dissolve the residue with 0.5 mL of **Reconstitution Buffer (**Solution 4**)**, mix fully.
4. Take 50 µL for analysis.
   Note: Sample dilution factor: 0.5, detection limit: 0.05 ppb

3.5 Pre-treatment of milk sample:

1. Centrifuge the milk at 4000 r/min for 10 min at 15°C, discard upper fat layer (If a refrigerated centrifuge is not available, chill sample to approx. 15°C 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 30s, then add 250 µL of **1.04 M $ZnSO_4$ Solution** (Solution 2) and vortex for 30s, centrifuge at 4000 r/min for 10 min at 15°C. If a refrigerated centrifuge is not available, chill sample to approx. 15°C prior to centrifugation.

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°C with nitrogen evaporators or water bath.
4. Dissolved the residue with 0.5 mL of **Reconstitution Buffer (**Solution 4**)**, mix fully.
5. Take 50 µL for analysis.

   Note: Sample dilution factor: 0.5, detection limit: 0.075 ppb

3.6 Pre-treatment 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 $Na_2Fe(CN)_5(NO)\cdot 2H_2O$ 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°C. If a refrigerated centrifuge is not available, chill sample to approx. 15℃ prior to centrifugation.
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°C with nitrogen evaporators or water bath.
4. Dissolve the residue with 0.4 mL of **Reconstitution Buffer (**Solution 4**)**, mix fully.
5. Take 50 µL for detection and analysis.

   Note: Sample dilution factor: 1, detection limit: 0.075 ppb

3.7 Pre-treatment of eggs sample:

1. Homogenize the sample use Homogenizer.
2. Weigh 1±0.05 g of homogenate sample into 50 mL centrifuge tube, add 8 mL of **Ethyl acetate** and vortex for 2 min. Centrifuge at 4000 r/min for 5 min.
3. Take 2 mL of the supernatant to another centrifuge tube, dry at 50-60°C with nitrogen evaporators or water bath.
4. Dissolve the residue with 2 mL of **N-hexane**, add 1 mL of **Reconstitution Buffer** (Solution 4), and vortex for 2 min. 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: 4, detection limit: 0.1 ppb

3.8 Pre-treatment of feed sample:

1. Homogenize the sample use Homogenizer.
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°C. If a refrigerated centrifuge is not available, chill sample to approx. 15℃ prior to centrifugation.
3. Take 3 mL of the supernatant to another centrifuge tube, dry at 50-60°C with nitrogen evaporators or water bath.
4. Dissolve the residue with 1 mL of **N-hexane**, added 1 mL of **Reconstitution Buffer** (Solution 4), and vortex for 30s. 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, detection limit: 0.025 ppb

3.9 Pre-treatment of water sample:

1. Take 0.5 mL of clear water sample into centrifuge tube (If the sample is cloudy, centrifuge at 4000 r/min for 10 minutes), add 0.5 mL of **2×Reconstitution Buffer** and vortex for 1 min.
2. Take 50 µL of clear liquid for analysis.
   Note: Sample dilution factor: 2, detection limit: 0.05 ppb

6. Assay Procedure

Bring all reagents and samples to room temperature (25°C) 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°C.

1. **Number:** Number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. **Standard and Samples must be tested 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. Vortex for 5s gently to mix thoroughly. Incubate at 25°C for 30 min away from direct sunlight.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 300 µL of **Wash Buffer** (Solution 5) to each well and immerse for 30s each time. Repeat wash procedure for 5 times, 30s intervals/time. Invert the plate and pat it against 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°C for 30 min away from direct sunlight.
5. **Wash:** repeat Step 3.
6. **Colour Development:** add 50 µL of **Substrate Reagent A** to each well, and then add 50 µL of **Substrate Reagent B**. Gently vortex for 5s to mix thoroughly. Incubate at 25°C in for 15 away from direct sunlight (The reaction time can be extended according to the actual colour change).
7. **Stop Reaction:** add 50 µL of **Stop Solution** to each well, vortex gently 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.

7. Data 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.

8. 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°C.
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 Microplate should be covered by plate sealer. Avoid the kit to strong light.
5. **Each reagent is optimized for use in the FSES0031. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other FSES0031 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns colour. When OD value of standard (concentration: 0) < 0.5 unit (A450nm < 0.5), it indicates the reagents are 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.

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Contact Details

Email: info@AsssayGenie.com

Web: www.AssayGenie.com