# Chloramphenicol (CAP) ELISA Kit

Technical Manual

(ELISA)

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## 1 Principle and Application

This kit adopts the method of indirect competitive enzyme-linked immunoassay (ELISA) to detect Chloramphenicol (CAP) in the sample such as tissue, liver, eggs, honey, milk, feed and water. The kit is composed of Microtiter Plate coated with coupled antigens, HRP enzyme conjugates, antibodies, standards and other supporting reagents. During the detection, with adding standards or samples, the CAP in the samples will compete with the coupled antigens to combine with anti-CAP antibodies. After adding enzyme conjugates, take coloration with TMB substrates. Absorbance value of the samples is a negative correlation with CAP content. Lastly, by comparing the obtained absorbance values with the standard curve, we can calculate the CAP content in the sample.

## 2 Technique Data

**2.1 Kit Sensitivity:** 0.025ppb (ng/mL)

**2.2 Reactive Mode:** 25°C, 30min ~ 30min ~ 15min

**2.3 Detection Limits:**

| Sample | Detection Limits |
|---|---|
| Tissue, liver, honey, milk | 0.0125ppb |
| Water | 0.05ppb |
| Eggs | 0.1ppb |
| Urine, serum, plasma, casings, feed, milk powder | 0.025ppb |

**2.4 Cross-reaction Rate:**

Chloramphenicol ..... 100%

Thiamphenicol, Florfenicol ..... <0.1%

**2.5 Sample Recovery Rate:**

| Sample | Recovery Rate |
|---|---|
| Tissue, liver | 85±20% |
| Eggs, honey, casings | 85±25% |
| Milk, feed, milk powder | 75±25% |
| Urine, serum, plasma | 70±20% |
| Water | 90±20% |

## 3 Composition of the Kit

| Reagent | Specification |
|---|---|
| Microtiter Plate | 8wells× 12strips |
| Standard: 0ppb, 0.025ppb, 0.075ppb, 0.225ppb, 0.675ppb, 2.025ppb | 1×1.0mL |
| High Standard (red cap): 100ppb | 1×1.0mL |
| Antibody solution (blue cap) | 1×5.5mL |
| HRP conjugate (red cap) | 1×11mL |
| Substrate Reagent A (white cap) | 1×6mL |
| Substrate Reagent B (black cap) | 1×6mL |
| Stop Solution (yellow cap) | 1×6mL |
| Concentrated Wash Buffer (20×)(white cap) | 1×40mL |
| Concentrated Reconstitution Buffer (2×) (yellow cap) | 1×50mL |
| Instruction | 1 |
| Adhesive Membrane | 1 |
| Sealed bag | 1 |

## 4 Materials Required but Not Supplied

**4.1 Equipment:** microplate reader, printer, grinder (for homogenizing solid samples), nitrogen evaporator, vortex mixer (for shake and mix), centrifuge, graduated transfer pipette, and balance with a division value of 0.01 g, constant temperature device;

**4.2 Micropipette:** single-channel (20-200μL and 100-1000μL), and multi-channel 300μL;

**4.3 Reagents:** Ethyl acetate, n-hexane, sodium acetate, acetic acid, sodium nitroprusside, β-glucuronidase, zinc sulfate.

## 5 Experimental preparation

*Restore all reagents and samples to room temperature (adjust to around 25°C) for more than 30 min before use. This is a crucial step to ensure there is no precipitation in the reagents.*

**5.1 Notice Before Sample Processing:**

Please note that the labware must be clean. Use disposable pipette tips to avoid contamination of interference results.

**5.2 Solution preparation:**

**Solution 1:** 0.36M Sodium Nitroprusside Solution (for milk and milk powder samples)

Dissolve 10.7 g of sodium nitroprusside in deionized water. Mix thoroughly and make up the volume to 100 mL.

**Solution 2:** 1.04M Zinc Sulfate Solution (for milk and milk powder samples)

Dissolve 29.8 g of zinc sulfate in deionized water. Mix thoroughly and make up the volume to 100 mL.

**Solution 3:** 0.1M Sodium Acetate Buffer (for urine samples)

Dissolve 2.4 g of sodium acetate in deionized water. Mix thoroughly and make up the volume to 500 mL. Add 1.2 mL of acetic acid and mix well.

**Solution 4:** Reconstitution Buffer (If the test sample is water, do not dilute it; use it directly.)

Dilute the Concentrated Reconstitution Buffer (2×) 2 times with deionized water (Reconstitution Buffer (2×): deionized water=1:1). It can be stored at 4 °C for one month.

**Solution 5:** Working Wash Buffer

Dilute the concentrated wash buffer (20×) by a factor of 20 (Concentrated wash buffer/Deionized water= 1: 19).

**5.3 Sample pretreatment steps:**

**5.3.1 Tissue, liver treatment.**

1) Weigh 3g ± 0.05g of homogenized sample into a 50mL centrifuge tube. Add 3mL of deionized water and shake for 30 seconds. Then add 6mL of ethyl acetate and shake for 2 minutes. Centrifuge at 4000 r/min at room temperature for 10 minutes.

2) Take 2 mL of the upper liquid layer and evaporate it to dryness under nitrogen or air at 50°C-60°C.

3) Dissolve the dried residue in 1mL of n-hexane, then add 0.5mL of Reconstitution Buffer (Solution 4). Shake for 30 seconds and centrifuge at 4000 r/min at room temperature for 5 minutes.

4) Discard the upper organic phase and take 50μL of the lower aqueous phase for analysis.

***Dilution times of the sample:0.5 Detection limits:0.0125ppb***

**5.3.2 Serum, plasma treatment.**

1) Transfer 1mL of serum or plasma into a centrifuge tube. Add 2mL of ethyl acetate and shake for 1 minute. Centrifuge at 4000 r/min at room temperature for 5 minutes.

2) Take 1 mL of the upper liquid layer and evaporate it to dryness under nitrogen or air at 50°C-60°C.

3) Dissolve the dried residue in 1mL of n-hexane, then add 0.5mL of Reconstitution Buffer (Solution 4). Shake for 30 seconds and centrifuge at 4000 r/min at room temperature for 5 minutes.

4) Discard the upper organic phase and take 50μL of the lower aqueous phase for analysis.

***Dilution times of the sample:1 Detection limits: 0.025ppb***

**5.3.3 Urine treatment.**

1) Transfer 2mL of urine into a centrifuge tube. Add 0.5mL of 0.1M Sodium Acetate Buffer (Solution 3) and mix. Then add 40μL of β-glucuronidase (prepared in deionized water to a concentration of 1000U/mL) and mix thoroughly. Incubate at 37°C overnight for hydrolysis.

2) After incubation, allow the solution to return to room temperature. Add 8mL of ethyl acetate and shake for 1 minute. Centrifuge at 4000 r/min at room temperature for 10 minutes. Take 4 mL of the upper liquid layer and evaporate it to dryness under nitrogen or air at 50°C-60°C.

3) Dissolve the dried residue in 1mL of Reconstitution Buffer (Solution 4) and mix thoroughly.

4)Take 50μL for analysis.

***Dilution times of the sample:1 Detection limits: 0.025ppb***

**5.3.4 Honey treatment.**

1) Weigh 2g ± 0.05g of honey into a centrifuge tube. Add 4mL of deionized water. Then add 4mL of ethyl acetate and shake for 2 minutes. Centrifuge at 4000 r/min at room temperature for 10 minutes.

2) Take 2 mL of the upper liquid layer and evaporate it to dryness under nitrogen or air at 50°C-60°C.

3) Dissolve the dried residue in 0.5mL of Reconstitution Buffer (Solution 4) and mix well.

4) Take 50μL for analysis.

***Dilution times of the sample:0.5 Detection limits:0.0125ppb***

***(Detection Limit: 0.0125 ppb; Quantification Limit: 0.05 ppb***

***Note: Due to potential interferences in some samples, a value of 0.05 ppb should be used as the positive determination threshold.)***

**5.3.5 Animal casing treatment.**

1) Wash the intestinal casing thoroughly and homogenize. Weigh 1g (±0.05g) of the homogenized sample and place it into a 50mL centrifuge tube. Add 10mL of ethyl acetate to the tube and shake for 2 minutes. Centrifuge at 4000 r/min at room temperature for 10 minutes.

2) Transfer 5mL of the upper layer (organic phase) to a clean container. Evaporate to dryness under nitrogen or air at 50°C-60°C.

3) Dissolve the dried residue in 1mL of n-hexane, then add 0.5mL of Reconstitution Buffer (Solution 4). Shake for 30 seconds and centrifuge at 4000 r/min at room temperature for 5 minutes.

4) Discard the upper organic phase and take 50μL of the lower aqueous phase for analysis.

***Dilution times of the sample:1 Detection limits: 0.025ppb***

**5.3.6 Milk treatment.**

1)Centrifuge the milk at 4000 r/min at room temperature for 10 minutes to remove the upper fat layer. Transfer 5mL of the defatted milk into a 50mL centrifuge tube.

Add 250μL of 0.36M Sodium Nitroprusside Solution (Solution 1) and shake for 30 seconds. Add 250μL of 1.04M zinc sulfate solution (Solution 2) and shake for 30 seconds. Centrifuge at 4000 r/min at room temperature for 10 minutes.

2)Transfer 2.2mL of the upper liquid (equivalent to 2mL of fresh milk) to another centrifuge tube. Add 4mL of ethyl acetate and shake for 2 minutes. Centrifuge at 4000 r/min at room temperature for 10 minutes.

3)Transfer 2mL of the upper liquid to a clean container. Evaporate to dryness under nitrogen or air at 50°C-60°C.

4)Dissolve the dried residue in 0.5mL of Reconstitution Buffer (Solution 4) and mix thoroughly.

5)Take 50μL for analysis.

***Dilution times of the sample:0.5 Detection limits: 0.0125ppb***

***(Detection Limit: 0.0125 ppb; Quantification Limit: 0.05 ppb***

***Note: Due to potential interferences in some samples, a value of 0.05 ppb should be used as the positive determination threshold.)***

**5.3.7 Milk powder treatment.**

1)Weigh 2g ± 0.05g of milk powder into a 50mL centrifuge tube. Add 10mL of deionized water to dissolve the milk powder. Add 1mL of 0.36M Sodium Nitroprusside Solution (Solution 1) and 1mL of 1.04M zinc sulfate solution (Solution 2), then shake for 30 seconds. Centrifuge at 4000 r/min at room temperature for 10 minutes.

2)Transfer 3.6mL of the upper liquid (equivalent to 0.6g of milk powder) to another centrifuge tube. Add 6mL of ethyl acetate and shake for 5 minutes. Centrifuge at 4000 r/min at room temperature for 10 minutes. Transfer 4 mL of the upper liquid to a clean container. Evaporate to dryness under nitrogen or air at 50°C-60°C.

3)Dissolve the dried residue in 0.4mL of Reconstitution Buffer (Solution 4) and mix thoroughly.

4)Take 50μL for analysis.

***Dilution times of the sample:1 Detection limits: 0.025ppb***

***(Detection Limit: 0.025 ppb; Quantification Limit: 0.075 ppb***

***Note: Due to potential interferences in some samples, a value of 0.075 ppb should be used as the positive determination threshold.)***

**5.3.8 Egg treatment.**

1)Weigh 1g ± 0.05g of homogenized egg sample into a 50mL centrifuge tube. Add 8mL of ethyl acetate and shake for 2 minutes. Centrifuge at 4000 r/min at room temperature for 5 minutes.

2)Transfer 2mL of the upper liquid to another centrifuge tube. Evaporate to dryness under nitrogen or air at 50°C-60°C.

3)Dissolve the dried residue in 2mL of n-hexane. Add 1mL of Reconstitution Buffer (Solution 4) and shake for 2 minutes. Centrifuge at 4000 r/min at room temperature for 5 minutes.

4)Remove the upper organic phase. Use 50μL of the lower aqueous phase for analysis.

***Dilution times of the sample:4 Detection limits: 0.1ppb***

**5.3.9 Feed treatment.**

1)Weigh 2g ± 0.05g of homogenized feed into a 50mL centrifuge tube. Add 2mL of deionized water. Add 6mL of ethyl acetate and shake for 2 minutes. Centrifuge at 4000 r/min at room temperature for 10 minutes.

2)Transfer 3mL of the upper liquid to another container. Evaporate to dryness under nitrogen or air at 50°C-60°C.

3)Dissolve the dried residue in 1mL of n-hexane. Add 1mL of Reconstitution Buffer (Solution 4) and shake for 30 seconds. Centrifuge at 4000 r/min at room temperature for 5 minutes.

4)Remove the upper organic phase. Use 50μL of the lower aqueous phase for analysis.

***Dilution times of the sample:1 Detection limits: 0.025ppb***

**5.3.10 Water treatment.**

1)Take 0.5mL of clear water sample (if the water sample is turbid, centrifuge at 4000 r/min at room temperature for 5 minutes to obtain a clear water sample) into a 1.5mL centrifuge tube. Add 0.5mL of Concentrated Reconstitution Buffer (2×) and shake for 1 minute.

2)Use 50μL of the prepared sample for analysis.

***Dilution times of the sample:2 Detection limits: 0.05ppb***

## 6 ELISA procedure

Place all reagents and samples to room temperature (adjust to around 25°C) for 30min. Gently shake the reagent bottles before use.

Take out the frame of the microplate along with the required number of wells. Then place the unused microplate wells into the sealed bag with the desiccant provided. Store the remaining kit in the refrigerator at 2-8°C.

**Step 1:** Number: Number the wells in sequence corresponding to the samples and standard, make 2-well parallel trials for each sample and standard, and record their locations.

**Step 2:** Sample Incubation: Add 50μL of standard or sample into each numbered well, then add 50μL of antibody solution into each well. Finally, cover the

Microtiter Plate with the adhesive membrane, shake gently by hand (or use a microplate shaker) for 5s and incubate for 30 min at 25°C in the dark.

**Step 3:** Washing: Uncover the adhesive membrane carefully, remove the liquid, pipette 350µL of Working Wash Buffer (Solution 6) to every well, let stand for 30 seconds then drain, repeat 5 times. Invert the plate and tap it against a thick absorbent paper (or lint-free cloth), with a soft towel placed underneath. (Bubbles that are not removed after tapping dry can be punctured with a clean pipette tip).

**Step 4:** Enzyme Incubation: Add 100µL of HRP conjugate into each well. Then cover the Microtiter Plate with the adhesive membrane, incubate for 30 min at 25°C in the dark.

**Step 5:** Washing: Same as step 3.

**Step 6:** Color: Add 50µL of Substrate Reagent A to each well. Then add 50µL of Substrate Reagent B per well. Shake gently by hand (or use a microplate shaker) for 5s, and allow to react for 15min at 25°C in the dark. (The reaction can be extended appropriately if the blue color is too pale.)

**Step 7:** Stop the reaction: Pipette 50µL of Stop Solution to each well, and shake gently by hand (or use a microplate shaker). The reaction would be stopped.

**Step 8:** Calculate: Determine the Optical Density (OD value; absorbance value) at 450nm (Reference wavelength 630nm) with a microplate reader. Finish this step within 10min after stop the reaction.

## 7 Interpretation of result

### 7.1 Calculate the percentage of absorbance value

$$\text{Percentage of absorbance value}(\%) = \frac{A}{A0} \times 100\%$$

A—the average OD value of the sample or standard;

A0—the average OD value of the 0ppb standard.

It is used to calculate the percentage absorbance of a standard or sample.

### 7.2 Draw the standard curve and calculate

Take absorbance percentage(A/A0) of standards as Y-axis and the corresponding log of standards concentration (ppb) as X-axis.

Draw the standard semi-log curves with X-axis and Y-axis.

Take absorbance percentage of samples substitute into standard curve, then can get the corresponding concentration from standard curve. **Last, the resulting concentration values multiplied by the corresponding dilution times is the actual concentration of CAP of samples.**

If professional analysis software of the kit is used for calculation, it is more convenient for accurate and rapid analysis of a large number of samples.

## 8 Attention

8.1 Before test, the reagents and samples should be balanced to room temperature (25°C). If below 25°C, it will lead to all the standard OD value on the low side.

8.2 In the washing process, dry wells may result in non-linear standard curves and undesirable reproducibility. Therefore, proceed to the next step immediately after washing.

8.3 Please mix the contents within the wells uniformly and wash the plate thoroughly. The reproducibility is largely determined by consistency of washing step.

8.4 During the incubation, cover microplates with adhesive membrane to avoid light.

8.5 Do not use kits that are overdue. Do not mix reagents with those from other lots.

8.6 Substrate Reagent A/B is colorless. If not, please discard.

8.7 If absorbance value of 0ppb is below 0.5（A450nm< 0.5 ), it means that the reagent may be metamorphic.

8.8Stop solution is corrosives, please avoid contact with skin.

8.9 As the OD values of the standard curve may vary according to the conditions of actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.

8.10 For the mentioned sample, fast and efficient extraction methods are included in the kit description. Please consult technical support for the applicability if other sample need to be tested.

8.11 The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS can be used for quantitative confirmation.

## 9 Storage conditions

The kit shall be stored at 2-8 °C. Avoid freezing.

Shelf Life: 12 months. The date of manufacture is presented in the label of the box.