# LSY-10007 Chloramphenicol(CAP) ELISA Test Kit (Tissue, egg)

**Chloramphenicol ELISA test Kit (Tissue, egg)**

Catalog No. LSY-10007

## 1. Principle

This test kit is based on theindirect competitive enzyme immunoassay for the detection of Chloramphenicol in the sample.The coupling antigenis pre-coated on the micro-well stripes. The Chloramphenicol in the sample and the coupling antigen pre-coated on the micro-well stripes compete for the anti-Chloramphenicol antibody. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the sample has a negative correlation with the Chloramphenicol in it. This value is compared to the standard curve and the Chloramphenicol concentration is subsequently obtained.

## 2. Technical specifications

Sensitivity: 15 ppt

Incubation Temperature: 25℃

Incubation Time: 30min～15min

Detection limit:

Tissue (method 1)7.5ppt

Tissue (method 2) 15ppt

Egg 15ppt

Recovery rate

Tissue, egg95±25%

Cross-reaction rate:

Chloramphenicol100%

Thiamphenicol< 0.1%

Florfeniol< 0.1%

## 3. Components

| | | | |
|---|---|---|---|
| 1 | Micro-well strips | 12 strips with 8 removable wells each | |
| 2 | 7× standard solution (1mL each) | 0ppt | 15ppt |
| | | 45ppt | 135ppt |
| | | 405ppt | 1215ppt<br>10ppb |
| 3 | Enzyme conjugate | 7ml | red cap |
| 4 | Antibody working solution | 7ml | blue cap |
| 5 | Substrate A | 7ml | white cap |

| | | | |
|---|---|---|---|
| 6 | SubstrateB | 7ml | black cap |
| 7 | Stop solution | 7ml | yellow cap |
| 8 | 20× concentrated washing buffer | 40ml | white cap |
| 9 | 2×concentrated redissolving solution | 50ml | transparent cap |

**4. Materials required but not provided**

1) Equipment' s: microplate reader, homogenizer, nitrogen-drying device, vortex, centrifuge, measuring pipets, and balance (sensibility reciprocalof0.01 g), incubator.

2) Micro pipettors: single-channel 20-200 µL, 100-1000 µL, adult-channel 30～300 µl.

3) Reagents: Ethyl acetate-hexane, NaOH, K2HPO4·3H2O, HCl.

**5. Sample pre-treatment**

Instructions:

The following points must be dealt with before the pre-treatment of any kind of sample:

1) Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents;
2) Before the experiment, each experimental equipment must be checked to be clean and should be re-cleaned if necessary, in order to avoid the contamination which interferes with the experimental results.

Solution preparation before sample pre-treatment

1) Sample redissolving solution: the2×concentrated redissolving solution is diluted with deionized water at 1:1.

**5.1 Tissue (Chicken, duck, pork, fish, shrimp, beef, lamb) Method 1**

1. Take 3± 0.05 g of the homogenized sample into a 50ml centrifuge tube. Firstly add 3 mL deionized water, then add 6mLethyl acetate, shake properly for 1 min, centrifuge at above4000 r/min at room temperature (20-25℃) for5 min.
2. Take 4mL of the supernatant, blow to dry by nitrogen in50-60℃.
3. Dissolve the dry residues in1 mL N-hexane, add 1 mL of the sample redissolving solution, mix for 30 seconds; centrifuge at above4000 r/min at room temperature (20-25℃) for 10 min, remove the up-layer organic phase.
4. Take50 µL of the down-layer for analysis.

Fold of dilution of the sample:0.5
(If cannot take 4ml supernatant during sample preparation, repeat centrifuge then to take solution, or add 9ml ethyl acetate then take 6ml supernatant blow to dry, the dilution factor is same)

**5.2 Tissue (Chicken, duck, pork, fish, shrimp, beef, lamb) Method 2**

1. Take2± 0.05 g of the homogenized sample into a 50ml centrifuge tube. Firstly add 3 mL deionized water, then add 6mLethyl acetate, shake properly for 1 min, centrifuge at above4000 r/min at room temperature (20-25℃) for5 min.
2. Take3mL of the supernatant, blow to dry by nitrogen in50-60℃.
3. Dissolve the dry residues in1 m LN-hexane, add 1 mL of the sample redissolving solution, mix for 30 seconds; centrifuge at above4000 r/min at room temperature (20-25℃) for5 min, remove the up-layer organic phase.
4. Take50 µL of the down-layer for analysis.

Fold of dilution of the sample: 1
(If cannot take 3ml supernatant during sample preparation, repeat centrifuge then to take solution, or add 10ml ethyl acetate then take 5ml supernatant blow to dry, the dilution factor is same)

**5.3 Other tissue samples with high fat content (kidney, liver, intestine, skin, heart, pork belly, etc.)**

1. Take2± 0.05 g of the homogenized sample into a 50ml centrifuge tube. Add10mL N-hexane, shake for3 min, centrifuge at above4000 r/min at room temperature (20-25℃) for5 min, discard N-hexane.
2. Add 6mLethyl acetate, shake for 1min, centrifuge at above4000 r/min at room temperature (20-25℃) for5 min.
3. Take3mL of the supernatant, blow to dry by nitrogen in50-60℃.
4. Dissolve the dry residues in1 mL N-hexane, add 1 mL of the sample redissolving solution, mix for 30 seconds; centrifuge at above4000 r/min at room temperature (20-25℃) for5 min, remove the up-layer organic phase.
5. Take50 µL of the down-layer for analysis.
   Fold of dilution of the sample: 1

### 5.4 Egg

1. Take2± 0.05 g of the homogenized sample into a 50ml centrifuge tube. Add 6mL ethyl acetate, shake properly for 1 min, centrifuge at above4000 r/min at room temperature (20-25℃) for5 min.
2. Take3mL of the supernatant, blow to dry by nitrogen in50-60℃.
3. Dissolve the dry residues in1 mL N-hexane, add 1 mL of the sample redissolving solution, mix for 30 seconds; centrifuge at above4000 r/min at room temperature (20-25℃) for5 min, remove the up-layer organic phase.
4. Take50 µL of the down-layer for analysis.

Fold of dilution of the sample: 1

(If cannot take 3ml supernatant during sample preparation, repeat centrifuge then to take solution, or add 10ml ethyl acetate then take 5ml supernatant blow to dry, the dilution factor is same)

## 6. ELISA procedures

### 6.1 Instructions

1. Bring all reagents and micro-wells trips to the room temperature (20-25 ℃) before use;
2. Return all reagentsto2-8 ℃ immediately after use;
3. The reproducibility of the ELISA analysis, to a large degree, depends on the consistency of plate washing. The correct operation of plate washing is the key point in ELISA the procedures;
4. For the incubation at constant temperatures, all the samples and reagents must avoid light exposure, and each microplate should be sealed by the cover membrane.

### 6.2 Operation procedures

1. Take out all the necessary reagents from the kit and place at the room temperature (20-25 ℃) for at least 30 min. Note that each reagent must be shaken to mix evenly before use.
2. Take the required micro-well strips and plate frames. Re-sealed the unused microplate, store at 2-8℃,not frozen.
3. Solution preparation: dilute 40 mL of the concentrated washing buffer (20 × concentrated) with the deionized water at 1:19 (1 part of

20Xconcentratedwashing buffer + 19 parts of deionized water), or prepare as quantity needed.

4. Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate, record their positions.
5. Add 50 µL of the sample or standard solution to separate duplicate wells; then add 50 µL enzyme conjugate into each well, at last add50 µL of antibody working solution into each well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, and incubate at 25 ℃ for 30 min.
6. Pour the liquid, wash the microplate with the diluted washing buffer at 250 µL/well for 4-5 times. Each time soak the well with the washing buffer for 15-30 sec, flap to dry with absorbent paper (if there are the bubbles after flapping, cut them with the clean tips).
7. Coloration: add50 µL of the substrate A solution and then50 µL of the B solution into each well. Mix gently by shaking the plate manually, and incubate at 25 ℃ for 15minatdark for coloration.
8. Determination: add50 µL of the stop solution into each well. Mix gently by shaking the plate manually. Set the wavelength of microplate reader at 450 nm to determine the OD value. (recommend to read the OD value at the dual-wavelength 450/630 nm within 5 min).

**7. Result judgment**

There are two methods to judge the results; the first one is through judgment, while the second is the quantitative determination.

Note that the OD value of the sample has a negative correlation with the content of Chloramphenicol.

**7.1 Qualitative determination**

The concentration range (ng/mL) can be obtained from the comparison the average OD value of the sample with that of the standard solution. Assuming that the OD value of the sample Ⅰ is0.3, and that of the sample Ⅱ is 1.0, while those of the standard solutions are as the followings: 2.243 for0ppt,1.816 for15ppt, 1.415for 45ppt,0.74 for 135ppt,0.313 for405ppt and 0.155 for 1215ppt, accordingly the concentration range of the samples 405 to1215ppt, and that of the sampleⅡ is 45 to135ppt.

## 7.2 Quantitative determination

The mean values of the absorbance values obtained for the average OD value (B) of the sample and the standard solution divided by the OD value (B0) of the first standard solution(0 standard) and subsequently multiplied by 100%, that is,

$$\text{Percentage of absorbance value} = \frac{B}{B0} \times 100\%$$

B—the average OD value of the sample or the standard solution

B0—the average OD value of the 0ng/mL standard solution

Draw the standard curve with the absorption percentages of the standard solution and the semilogarithmic values of the Chloramphenicol standard solution(ng/mL) as Y-and X-axis, respectively. Read the corresponding concentration of the sample from the standard curve by incorporating its absorption percentage into the standard curve. The resulting value is subsequently multiplied by the corresponding dilution fold, thus finally obtaining the Chloramphenicol concentration in the sample.

Using the professional analyzing software of this kit will be more convenient for the accurate and rapid analysis of a large amount of samples. (Please contact us for this software).

## 8. Precautions

1. The room temperature below 25℃ or the temperature of the reagents and the samples being not returned to the room temperature (20-25 ℃) will lead to a lower standard OD value.
2. Dryness of the microplate in the washing process will be accompanied by the situations including the non-linear standard curves and the undesirable reproducibility.
3. Mix every reagent and reaction mixture evenly and wash the microplate thoroughly, otherwise there willbe the undesirable reproducibility.
4. The stop solution is the2 M sulfuric acid solution, avoid contacting with the skin;
5. Put the unused microplate into anauto-sealing bag to re-seal it. The standard substance and the colourless color former is light sensitive, and thus they cannot be directly exposed to the light.
6. Do not use the kit exceeding its expiry date. The use of diluted or adulterated reagents from the kits will lead to the changes in the sensitivity and the detecting OD values. Do not exchange the reagents from the kits of different lot numbers to use.

7. Discard the colouration solution with any color that indicates the degeneration of this solution. The detecting value of the standard solution 1 (0 ppb) of less than 0.5 indicates its degeneration.
8. The optimum reaction temperatureis25 ℃,and too high or too low temperatures will result in the changes in the detecting sensitivity and OD values.

**9. Storage and expiry date**

Storage: store at2-8 ℃, not frozen.

Expiry date: 12 months; date of production is on the box.
Note: If the Vacuum package of microplate has leakage, it is still valid to use, do not affect the test result, be relax to use.