Chloramphenicol ELISA test Kit

1. Principle

This test kit is based on the competitive enzyme immunoassay for the detection of Chloramphenicol in the sample. The coupling antigen is 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: 0.05 ppb

Detection limit:

Intestine.....0.1 ppb
Honey.....0.1 ppb
Milk.....0.05 ppb
Egg.....0.1 ppb
Urine, serum.....0.1 ppb
Chicken/liver, pork/liver, fish, shrimp.....0.025 ppb

Recovery rate

Chicken/liver, pork/liver, fish, shrimp..... 80±10%
Honey,intestine..... 70±10%
Milk,egg..... 85±15%
Urine,serum..... 70±10%

Cross-reaction rate:

Chloramphenicol 100%
Thiamphenicol..... < 0.1%
Florfeniol ..... < 0.1%

3. Components

1） Micro-well strips: 12 strips with 8 removable wells each
2） 6× standard solution (1 mL each): 0 ppb, 0.05 ppb, 0.15 ppb, 0.45 ppb, 1.35 ppb and 4.05 ppb
3） Enzyme conjugate (12 mL) .....red cap
4） Concentrated antibody working solution (1 mL)..... blue cap
5） Substrate A solution (7 mL) .....white cap
6） Substrate B solution (7 mL).....black cap
7） Stop solution (7 mL) .....yellow cap
8） 20× concentrated washing buffer (40 mL) .....white cap
9） 2× concentrated redissolving solution (50 mL) .....transparent cap

4. Materials required but not provided

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1) **Equipments:** microplate reader, homogenizer, nitrogen-drying device, vortex, centrifuge, measuring pipets, and balance(a sensibility reciprocal of 0.01 g).
2) **Micropipettors:** single-channel 20-200 µL, 100-1000 µL, and multi-channel 250 µL.
3) **Reagents:** Ethyl acetate, Acetonitrile (CH_3CN), N-hexane, $Na_2Fe(CN)_5 \cdot NO \cdot 2H_2O$ for milk sample, $ZnSO_4.7H_2O$ for milk sample, Beta-Glucuronidase (Merck, Art. No. 4114) for urine, CH_3COONa, CH_3COOH,deionized water

5. Sample pre-treatment

Instructions

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

1) This product can detect the following tissue samples:animal tissue,poultry,and aquatic tissue, eg:chicken,duck, beef,rabbit,fish,shrimp,etc.

2) Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents;

3) Before the experiment, each experimental equipment must be clean and should be re-cleaned if necessary, in order to avoid the contamination that interferes with the experimental results.

Solution preparation before sample pre-treatment

1) *C solution* (for milk sample): dissolve 0.36 g $Na_2Fe(CN)_5 \cdot NO \cdot 2H_2O$ in the deionized water to 100 mL .
2) *D solution* (for milk sample): dissolve 28.8 g $ZnSO_4.7H_2O$ in deionized water to 100 mL.
3) pH 4.8 100 mM CH_3COONa buffer(for urine sample): add 2.4 g CH_3COONa and 1.2 mL acetic acid in deionized water to 500 mL, and adjust pH to 4.8.
4) Acetonitrile-H_2O solution: $V_{acetonitrile}$:V_{H2O} = 84:16
5) The 2×concentrated redissolving solution is diluted with deionized water at 1:1 (1 mL concentrated redissolbing solution + 1 mL deionized water).

5.1 Tissue(Shrimp, fish, meat/liver)

1. Homogenize the sample .
2. Take 3± 0.05 g of the homogenized sample into tube. Firstly add 3 mL deionized water and mix evenly, then add 6mL ethyl acetate, shake properly for 5 min, centrifuge at above 4000 r/min at room temperature (20-25℃) for 10 min.
3. Take 4mL of the supernate (equivalent to 1 g sample), and evaporate to dryness by nitrogen in 50-60℃ waterbath.
4. Dissolve the dry residues in 1 mL N-hexane, add 1 mL of the diluted redissolving solution, shake vigorously for 30 seconds; centrifuge at above 4000 r/min at room temperature for 5 min.
5. Take 50 µL of the lower for analysis.

Fold of dilution of the sample: 1

5.2 Serum, plasma

1. Transfer 1 mL sample into tube, add 2 mL ethyl acetate, shake properly for 1 min.
2. Centrifuge at 4000 r/min at room temperature for 5 min.

3. Transfer ethyl acetate layer (upper layer) into a new vessel and evaporate to dryness by nitrogen in 56 ℃ waterbath.
4. Dissolve the dry residue with 1 mL diluted redissolving solution for 30 seconds.
5. Take 50 µL for further analysis.

Fold of dilution of the sample: 1

5.3 Urine

1. Transfer 2 mL urine into centrifuge tube, mix with 0.5 mL100 mM CH_3COONa buffer(pH 4.8).
2. Add 40 µL of the Beta-Glucuronidase into the diluted urine, hydrolyze at 37 ℃ for 2 h (or over night).
3. Return to room temperature (20-25 ℃), add 8 mL ethyl acetate, mix properly for 1 min.
4. Centrifuge at 4000 r/min at room temperature (20-25 ℃) for 10 min, take out 4 mL of the upper layer and evaporate to dryness by nitrogen at 50-60 ℃.
5. Dissolve the dry residue in 1 mL of the diluted redissolving solution for 30 seconds.
6. Take 50 µL for further analysis.

Fold of dilution of the sample: 1

5.4 Honey

1. Put 2.0 ± 0.05 g honey into centrifuge tube, diluted in 4 mL of the deionized water.
2. Add 4 mL ethyl acetate, shake upside and down for 5 min.
3. Centrifuge at 4000 r/min at room temperature (20-25 ℃) for 10 min.
4. Transfer 2 mL of the ethyl acetate layer (upper layer) into a new vessel, evaporate to dryness by nitrogen at 50-60 ℃.
5. Dissolve the dry residue in 0.5 mL of the diluted redissolving solution for 30 seconds.
6. Take 50 µL for further analysis.

Fold of dilution of the sample: 1

Detection limit: 0.5 ppb **Quantitative limit:** 0.025 ppb

Note: Recommend 0.1 ppb as value of cut off for positive sample.

5.5 Intestine

1. Homogenize the sample(note that dry sample must be cut into less than 5 mm pieces; damp sample must be washed to eliminate salt for 20 min with the deionized water, reduce water and then homogenize).
2. Weigh 1.0±0.05 g of the homogenized sample into centrifuge tube, add 10 mL ethyl acetate, shake properly for 5 min, centrifuge at above 4000 r/min at room temperature (20-25 ℃) for 10 min.
3. Take out 5 mL of the upper layer (equivalent to 0.5 g sample), blow to dry with nitrogen at 50-60℃.
4. Dissolve the dry residues in 1 mL N-hexane, add 0.5 mL of the diluted redissolving solution, shake strongly for 30 seconds; centrifuge at above 4000 r/min at room temperature for 5 min.
5. Discard upper layer, take 50 µL of the lower for further analysis.

Fold of dilution of the sample: 1

5.6 Milk and milk powder

First method for milk sample

1. Centrifuge at above 4000 r/min at 10 ℃ for 10 min, remove fat (upper layer).
2. Put 5 mL sample without fat into centrifuge tube, add 150 µL *C solution* and appear precipitation, shake properly, then add 150 µL *D solution, mix properly*.
3. Centrifuge at above 4000 r/min at 15 ℃ for 10 min, take upper layer.
4. Dissolve the upper layer in the diluted redissolving solution at 1:1, mix for 30 seconds.
5. Take 50 µL for further analysis.

Fold of dilution of the sample: 2

Detection limit: 0.1 ppb **Quantitative limit:** 0.15 ppb

Note: if appear turbidity after centrifuge, repeat the step of precipitation.

Second method for milk sample

1. Put 5 mL milk removed fat into centrifuge tube.
2. Add 250 µL *C solution* and 250 µL *D solution*, mix thoroughly, centrifuge at above 4000 r/min at 4-12 ℃ for 10 min. if centrifuge of constant temperature is not avaible, chill sample temperature to approx 8 ℃, then centrifuge.
3. Transfer 2.2 mL upper layer (equivalent to 2 mL sample) into a new vessel, add 4 mL ethyl acetate, shake upside down for 5 min.
4. Centrifuge at above 4000 r/min at room temperature (20-25℃) for 10 min.
5. Transfer 2 mL of the ethyl acetate (upper layer, equivalent to 1 mL sample), blow to dry with nitrogen at 60℃ competely.
6. Dissolve dry residues in 0.5 mL of the diluted redissolving solution .
7. Take 50 µL for further analysis.

Fold of dilution of the sample: 0.5

Detection limit: 0.025 ppb **Quantitative limit:** 0.05 ppb

Note: Recommend standards 3 (0.15 ppb) as value of cut off for positive result, because negative sample causes interference, sometimes value of interference between standards 2 and standards 3.

Milk powder sample

1. Weigh 2.0 ± 0.05 g milk powder into centrifuge tube, add 10 mL of the deionized water , shake thoroughly to dissolve.
2. Add 1 mL *C solution* and *1 mL D solution*, mix thoroughly, centrifuge at above 4000 r/min at 4-12 ℃ for 10 min. if centrifuge of constant temperature is not avaible, chill sample temperature to approx 8 ℃, then centrifuge.
3. Transfer 3.6 mL of the upper layer (equivalent to 0.6 g sample) into a new vessel, add 6 mL ethyl acetate, shake upside down for 5 min.
4. Centrifuge at above 4000 r/min at room temperature (20-25 ℃) for 10 min.
5. Transfer 4 mL of the ethyl acetate (upper layer, equivalent to 0.4 g sample), blow to dry with nitrogen at 60℃ competely.

6. Dissolve dry residues in 0.4 mL of the diluted redissolving solution for 30 seconds .
7. Take 50 μL for further analysis.

Fold of dilution of the sample: 1

Detection limit: 0.05 ppb **Quantitative limit:** 0.15 ppb

Note: Recommend standard 3 as value of cut off for positive result, because negative sample cause interference, sometimes value of interference between standards 2 and standards 3.

5.7 Egg

1. Homogenize the sample (whole egg or yolk).
2. Weigh 3.0 ± 0.05 g of the homogenized sample, add 9 mL of the acetonitrile-H_2O solution ($V_{acetonitrile}$:V_{H2O} = 84:16), shake for 5 min, centrifuge at above 4000 r/min at 15 ℃ for 10 min.
3. Transfer 3 mL of the upper layer into a centrifuge tube, add 3 mL of the deionized water, mix properly, then add 4.5 mL ethyl acetate, mix properly for 5 min, centrifuge at above 4000 r/min at 15 ℃ for 10 min.
4. Transfer the organic phase (upper layer) into a new centrifuge tube, blow to dry with nitrogen at 50℃.
5. Dissolve dry residues in 1 mL N-hexane, add 2 mL of the diluted redissolving solution, mix peoperly for 30 seconds, centrifuge to remove N-hexane.
6. Take 50 μL for further analysis.

Fold of dilution of the sample: 2

Detection limit: 0.1 ppb **Quantitative limit:** 0.3 ppb

6. ELISA procedures

6.1 Instructions

1 Bring all reagents and micro-well strips to the room temperature (20-25 ℃) before use;

2 Return all reagents to 2-8 ℃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 distilled or deionized water at 1:19 to 800 mL (or just to the required volume) for use.
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. Dilute the Concentrated antibody working solution with the diluted redissolving solution at 1:10

(1 mL concentrated antibody + 10 mL diluted redissolving solution).

6. Add 50 µL of the sample or standard solution to separate duplicate wells; and add 50 µL of the diluted 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.
7. Pour the liquid, wash the microplate with the 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).
8. Add 100 µL enzyme conjugate into every well, seal the microplate with the cover membrane, and incubate at 25 ℃ for 30 min, continue as described in 7.
9. Coloration: add 50 µL of the substrate A solution and then 50 µL of the B solution into each well. Mix gently by shaking the plate manually, and incubate at 25 ℃for 15 min at dark for coloration.
10. Determination: add 50 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 the rough 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 is 0.250, and that of the sample Ⅱ is 0.720, while those of the standard solutions are as the followings: 1.610 for 0 ppb, 1.380 for 0.05 ppb, 1.100for 0.15 ppb, 0.620 for 0.45 ppb, 0.289 for 1.35 ppb and 0.108 for 4.05 ppb, accordingly the concentration range of the sample Ⅰ is 1.35 to 4.05 ppb, and that of the sample Ⅱ is 0.15 to 0.45 ppb.

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,

$$\text{Percentage of absorbance value} = \frac{B}{B_0} \times 100\%$$

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

B_0—the average OD value of the 0ng/mL standard solution

Draw the standard curve with the absorption percentages of the standard solution and the semilogarithm 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 20 ℃ 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 will be the undesirable reproducibility.
4. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin;
5. Put the unused microplate into an auto-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 temperature is 25 ℃, 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 at 2-8 ℃, not frozen.

Expiry date: 12 months; date of production is on the box.

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Green Earth depends on everyone' efforts

"Build of green Earth needs the cooperation of you and me"

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