UC Biodevices

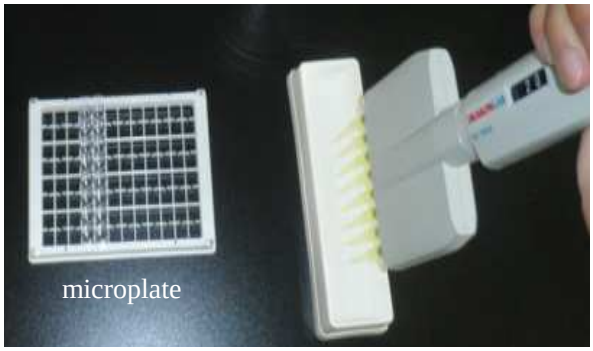
ELISA Kit for Detection of Chloramphenicol

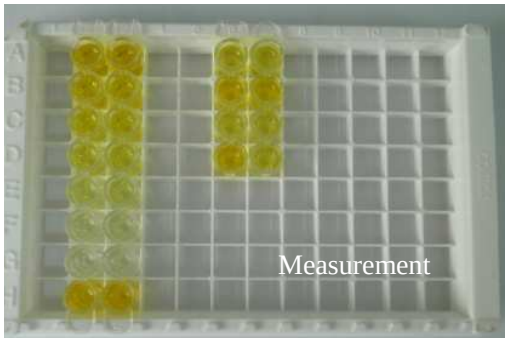
(Product Number: 5501E211)

（Chloramphenicol has blood system toxicity）

INSTRUCTION MANUAL

(v. 2.00)





1. Introduction

Chloramphenicol (CAP) is a broad-spectrum bacteriostatic antibiotic, obtained originally from the bacterium Streptomyces venezuelae. Due to potential side effects in humans, the drug is not recommended for the treatment of minor diseases, but is reserved for the treatment of serious infections. In veterinary medicine, CAP has been shown to be a highly effective, well-tolerated antibiotic; the potential side effects observed in humans have not been reported in animals. However, because of its toxicity in humans, the use of CAP in animal-derived foods, including honey and milk has been strictly regulated. The European Union (EU) has defined a maximum residue limit (MRL) for CAP in food of animal origin at a level of 0.3μg/kg, while China has an MRL level of 0.5 μg/kg. Currently, ELISA detection of chloramphenicol residues as a screening method has been widely used.

This manual establishes procedures on detection of chloramphenicol in food. The test is a competitive direct ELISA that provides exact concentrations in parts per billion (ppb). Chloramphenicol in samples and standards competes with enzyme-label conjugated for the antibody binding sites. After wash steps, a substrate solution is added and generates blue color product with assistance of the bound enzyme conjugate. To make quantitative test, a stop solution is then added into the blue color solution to stop the enzymatic reaction while causing the solution color change from blue to yellow for measurement. The intensity of the solution color is inversely proportional to the concentration of chloramphenicol in the samples and standards.

Assay Sensitivity, Precision, Accuracy

1) Sensitivity: 0.05ppb
2) Precision：CV <10%
3) Accuracy：60-120％ average recovery rate

2. Kit Contents

1) 1×Microtiter plate (8 wells×12 strips): precoated with antibodies to mouse IgG.
2) 6×CAP standard solutions (1ml each): 0 ng/ml, 0.05ng/ml,0.1ng/ml,0.5ng/ml,2.0ng/ml,10ng/ml
3) 1×CAP Enzyme Conjugate (ready-to-use): 10ml.
4) 1×Washing buffer(10×): 50ml
5) 1×CAP diluent buffer: 50ml
6) 1×Substrate solution (TMB) (ready-to-use): 17ml.

7) 1×Stop solution (ready-to-use): 7ml

8) 1×Instruction Manual.

9) 1×QC report.

Materials and reagents required but not provided:

1) Microtitre plate reader with 450nm filter.
2) Centrifuge
3) Homogenizer
4) 20-200µl and 200-1000µl precision micropipette.
5) 30-500µl 8-channel micropipette.
6) Microwell plate mixer.
7) Timer.
8) Nitrogen blowing device
9) Ethyl acetate
10) N-hexane or normal heptane
11) Wright'sI：0.36M potassium ferrocyanide
12) Wright'sII：1.04M zinc sulfate heptahydrate

3. Preparation of Working Solutions

- **CAP standard solutions**: ready to use.
- CAP **enzyme conjugate**: ready to use.
- CAP **diluent buffer**: ready to use.
- **10×wash buffer**: dilute 10x with pure water (1+9). (e. g. 20.0ml 10×Wash buffer +180.0 ml pure water, sufficient for 4 microtiter strips 48 wells).
- **TMB**: ready to use.
- **Stop solution**: ready to use.

4. Preparation of Samples

4.1 Meet

a. Take 3 g of the homogenized sample into a centrifugal screw cap vial, add 6ml Ethyl acetate and shake 10 min.
b. Centrifuge: 10 min, 3000 g, room temperature.
c. Take 2 ml of the above supernatant into a vial. Dry it by blowing it with Nitrogen gas via a blowing device (50℃).
d. Add 1 ml of N-hexane and 1 ml of diluent buffer into the above vial containing the dried substance. Then, mix them with a vortex mixer for 1 min.
e. Centrifuge the above mixture at 3000 g for 10 minutes. The mixture is separated into top hexane layer, middle emulsion layer and bottom aqueous layer
f. Aspirate the top hexane and middle emulsion layers, and keep the bottom aqueous extract. Take 100µl of the above exact when testing.

The dilution factor is 1.

4.2 Honey

a. Transfer 2 g of the sample into a centrifugal screw cap vial, add 4ml DI water and 4ml Ethyl acetate then shake 10 min.
b. Centrifuge: 10 min, 3000 g, room temperature.
c. Take 2 ml of the above supernatant into a vial. Dry it by blowing it with Nitrogen gas via a blowing device (50℃).
d. Add 1 ml of diluent buffer into the above vial with the dried substance and mix them with a vortex mixer for 1 min.
e. Take 100µl of the above solution when testing.

The dilution factor is 1.

4.3 Eggs

a. Make a homogeneous sample with a homogenizer.
b. Transfer 3 g of the homogenized sample into a centrifugal screw cap vial, add 6ml Ethyl acetate and shake 10 min.
b. Centrifuge: 10 min, 3000 g, room temperature.
c. Take 2 ml of the above supernatant into a vial. Dry it by blowing it with Nitrogen gas via a blowing device (50℃).
d. Add 1 ml of N-hexane and 1 ml of diluent buffer into the above vial containing the dried substance. Then, mix them with a vortex mixer for 1 min.
e. Centrifuge the above mixture at 3000 g for 10 minutes. The mixture is separated into top hexane layer, middle emulsion layer and bottom aqueous layer
f. Aspirate the top hexane and middle emulsion layers, and keep the bottom aqueous extract. Take 100µl of the above exact when testing.

The dilution factor is 1.

4.4 Milk

a. Centrifuge: 10 min, 3500 g, room temperature.
b. Aspirate the top layer of fat.
c. Transfer 5mL of the skimmed milk (bottom layer) into a centrifugal screw cap vial, add 150µl Wright'sI and 150µl Wright'sII， mix them thoroughly.
d. Centrifuge: 10 min, 3500 g.(If the supernatant is still turbid, add 150µl Wright'sI and 150µl Wright'sII and mix them thoroughly before the centrifugation）
e. Take 1 ml of the supernatant into a vial containing 1 ml diluent buffer, then mix them thoroughly.
f. Take 100µl of the above solution when testing.

The dilution factor is 2.

4.5 Urine

a. Take 2 ml of sample
b. Centrifuge: 10 min, 3000 g, room temperature.
c. Place 0.5ml of the supernatant into a vial containing 2ml of diluent buffer, mix them thoroughly.
d. Take 100µl of the above solution when testing.

The dilution factor is 5 .

5. Immunoassay Procedure

1) Allow reagents, microwells, and sample extracts to reach room temperature prior to perform the

test.

2) Insert a sufficient number of wells into the microwell holder for all standards and samples to be tested.
3) Using a new pipette tip for each standard and sample, pipette 100µl of standards and prepared sample to separate wells.
4) Add 50µl of enzyme conjugate into each well.
5) Incubate 60 minutes at 37℃, or 120 minutes at room temperature.
6) Dump the contents of the wells. Turn the wells upside down and tap out on a paper towel until the remaining liquid has been removed.
7) Add 250µl wash solution into each of the testing wells, empty all the wells by inverting the microplate, repeat this step 4 times. Finally, remove the residual droplets by vigorous knocking the microplate on a paper towel.
8) Add 150µl of TMB to each well.
9) Incubate for 12-15 min. at 37℃. Cover the wells with a cover (e.g. paper towel) to protect them from direct light source.
10) Add 50µl of stop solution to each well. Mix them thoroughly.
11) The absorbance is measured using a microplate reader with a 450 nm filter

6. Calculation

Divide the mean absorbance value of standards or samples (B) by the mean absorbance value of the Maximum Binding (B0) and multiply by 100. Maximum binding is thus made equal to 100% and the absorbance values are quoted in percentages:

$$\frac{\text{absorbance standard (or sample)}}{\text{absorbance maximum binding}} \times 100 = B/B0\ \%$$

7. Results

Since the samples have been diluted prior to assay, the concentrations in ng/ml read from the standard curve must be multiplied by the respective dilution factor to obtain the effective chloramphenicol concentration in samples expressed in ppb (ng/ml or ng/g). The dilution factors are 1 for meat, honey, and eggs; 2 for milk; 5 for urine; respectively.

8. Cautions

1) Each reagent is optimized for use in the UC Biodevices chloramphenicol Plate Kit. Do not substitute regents from any other manufacturer.
2) Avoid contacting the reagents (particularly standard solutions and stop solution) with the skin

when without wearing gloves

3) If ambient temperature is lower than 20℃ or the temperature of the reagents and standard solutions is less than the ambient temperature (e.g., 20- 25℃) when used, the testing results may have negative bias (lower than it should be).
4) If it takes too long time to dry the washed microplate during the washing steps, the results may demonstrate nonlinear calibration plot and poor repeatability, thus once the microplate is dried after the washing steps, the following step in the testing protocol should be immediately implemented to prevent the undesirable occurrence.
5) The standard solutions and chromogenic solution contain light-sensitive substances, thus the solutions should not be exposed under direct light.
6) To gain excellent results, any solutions should be thoroughly mixed before added on microplate and the microplate should be completely cleaned in between each step.
7) The stop solution is a very strong acidic solution, so users should avoid the solution directly contacting with skin.
8) Neither use expired reagents, nor dilutes reagents.
9) Do not interchange individual reagents between kits with different lot numbers.
10) The kit should be stored at 2-8℃ but not be frozen. Place any unused microwells to their original foil bag and reseal them together with the desiccant provided.

If there is difficulty to get great results by following the manual carefully, the matrix for the testing samples may be the cause. Please contact us and we can provide helps to develop a new assay kit or kits for your specific applications.

9. Contact Information

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