CAP(Chloramphenicol) ELISA Kit (Combination Ⅱ)Manual

(Art. No.: KITW001.ZH.02)

【Product Description】

This kit (Art. No.: KITW001.ZH.02) provides a enzyme-linked immunosorbent assay (ELISA) for the quantitative analysis of CAP (Chloramphenicol) in tissue specimen. Besides the specificity of the kit has evaluated by analyzing the cross-reactivities to corresponding substances. The kit is a convenient, rapid, and sensitive assay for on-site large-scale screening of CAP in tissue specimen.

【Technical Specifications】

1. **Sensitivity**: 0.02 ng/g or ppb.
2. **Detection Limit**: 0.1 ng/g or ppb;Fish,Shrimp,Chicken,Duck,Pork.
3. **Recovery rate**: 60%-120%.
4. **Specificity:**

   Chloramphenicol (CAP)----------100%

   Chloramphenicol Succinate----------121%

   Chloramphenicol Base----------<5.0%

   Florfenicol----------<0.1%
5. **Precision**： CV < 10%

【Components provided】

No.	Component	Quantity
1	Standards： 0 ppb 0.02 ppb 0.06 ppb 0.18 ppb 0.54 ppb 1.62 ppb	 1 vial (1.5 mL) 1 vial (1.5 mL) 1 vial (1.5 mL) 1 vial (1.5 mL) 1 vial (1.5 mL) 1 vial (1.5 mL)
2	CAP standard solution (10 ppb)	1 vial (1 mL)
3	Microtiter plate	1 x 96 well plate (8 wells x 12 strips)
4	Enzyme Conjugate Solution Concentrate (5×)	1 bottle (2 mL)
5	Wash Solution Concentrate (20×)	1 bottle (25 mL)

5	Substrate A Solution	1 bottle (7 mL)
6	Substrate B Solution	1 bottle (7 mL)
7	Stop Solution	1 bottle (7 mL)
8	Self-sealing Bag	1pc
9	Adhesive Plate Sealer	1pc
10	Instruction Manual	1 book

【Storage instructions】

1. Store the kit/reagents at 2-8℃(35.6-46.4℉). The shelf life is 12 months when properly stored.
2. Return unused micro-wells to their original foil bag and reseal them together with the provided desiccant and store at 2-8℃ (35.6-46.4℉).

【Materials Required but not Provided】

1. Equipment:

a. Pipettes and tips
b. Microtiter plate reader (450 nm)
c. Precision balance (accuracy:0.01 g)
d. Vortex mixer
e. Incubator(controllable temperature: 75℃)
f. Centrifuge (4000 g)
g. Nitrogen Evaporator
h. Timer

2. Reagent:

a. Ethyl Acetate (AR)
b. Normal Hexane (AR)
c. Distilled Water

【Reagent Preparation】

1. **Wash Solution:** Mix 1 volume of **Wash Solution Concentrate (20×)** with 19 volumes of distilled water.
2. **Enzyme Conjugate Solution:** Mix 1 volume of **Enzyme Conjugate Solution Concentrate (5×)** with 4 volumes of **Sample Diluent** (provided in **Combination Ⅱ Reagent Kit**). Use it within 5min.
3. **TMB Substrate:** Mix **Substrate A Solution** with equal volume of **Substrate B Solution**. Use it

within 5 min.(Do not put any substrate back to the original container to avoid any potential contamination. Any substrate solution exhibiting coloration is indicative of deterioration and should be discarded. Covering the microtiter plate while incubating is recommended.)

【Test Procedure】

1. Insert appropriate number of strips into a micro well holder. Record standard and sample positions. It is suggested that all standards and samples be run in duplicate.
2. Add 100 μL of each standard or sample (prepared according to the manual provided with Combination Ⅱ Reagents Kit) in duplicate into different wells.
3. Add 50 μL of **Enzyme Conjugate Solution** to each well.
4. Cover it with **Adhesive Plate Sealer**, and mix gently by rocking the plate for 10 s. Incubate for 30 min at room temperature(25±2℃).
5. Open the **Adhesive Plate Sealer** and dump the liquid in wells.
6. Wash the plate 4 times with 260 μL of **Wash Solution**.
7. After the last wash, tap dry the plate on paper towels.
8. Add 100 μL of **TMB Substrate** solution into each well.
9. Repeat step 4 but incubate for 15-20 min at room temperature.
10. Open the **Adhesive Plate Sealer** and add 50 μL of **Stop Solution** into each well. Mix gently by rocking for 10 s.
11. Read the plate with a microtiter plate reader using a 450 nm filter within 5 min.(Air bubbles should be eliminated prior to reading plate as they may affect analytical result.)

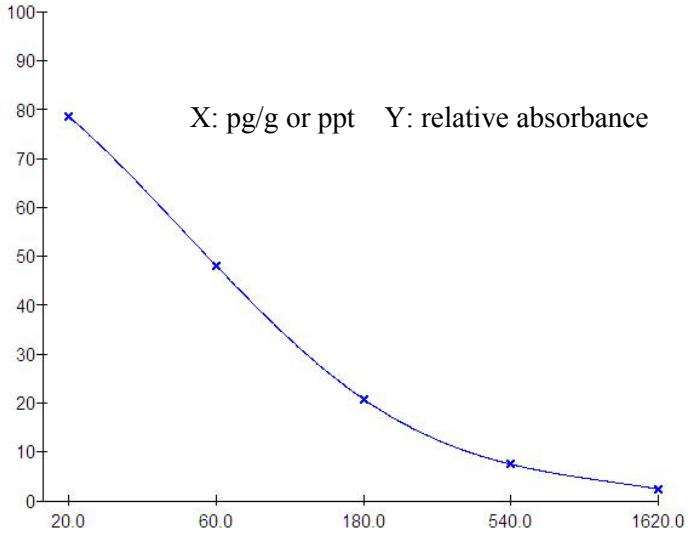
【Results interpretation 】

1. Using the OD values (relative absorbance) expressed as a percentage of the OD of the zero standard, construct a dose-response curve using the five standards. Since the amount of analyte in each standard is known, the unknowns in the sample can be measured by interpolation from the standard curve.

$$\text{Relative absorbance } (\%) = \frac{\text{absorbance standard (or sample)}}{\text{absorbance zero standard}} \times 100$$

2. To obtain the analyte concentration actually contained in a sample, the concentration read from the standard curve must be further multiplied by the corresponding dilution factor. The following is a typical CAP standard curve of the kit.

CAP Standard Curve



Standard（ng/g or ppb）	0	0.02	0.06	0.18	0.54	1.62
Relative absorbance	2.178	1.714	1.048	0.453	0.163	0.053

3. This kit is only for screening test, positive result should be further confirmed with other method(HPLC, LC, MS).

【Notes】

1. Please read the manual carefully before assay.
2. Do not use the kit past the expiration date.
3. Do not intermix micro-wells and reagents from different kits or lots.
4. All reagents should be restore up to room temperature before use (1-2 hours at 20-25℃).
5. Incubation times and temperatures of assay should be controlled as precisely as possible. Be consistent when adding standards and samples.
6. To avid time lag, please use 8-channel pipette when adding Enzyme Conjugate Solution, Wash Solution, and the like.
7. Please make sure the samples are fresh or properly stored. In general, samples should be refrigerated at 2-4℃ for no more than 1-2days.
8. Any metallic substance should be avoided to contain or agitate reagents.
9. Shake the reagent well before use especially for solutions with crystal liberation. Avoid air bubbles when mixing.
10. Avoid running assays under or near air vents, as this may cause excessive cooling, heating and/or evaporation.

11. The stop solution contains strong acid substances. Please wash by water immediately if it splashes on clothes or skin.
12. Please do not reuse pipette tips.
13. All wastes should be properly decontaminated prior to disposal. Dispose of contents in accordance with local, regional, and national regulations.

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