Version 09-04/24

User's Manual

Chloramphenicol ELISA Kit

REF **DEIA6881**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

Chloramphenicol (CAP) ELISA Test Kit is a competitive enzyme immunoassay for the quantitative analysis of chloramphenicol in fish, shrimp, eggs, honey, meat (beef, chicken and pork), milk, milk powder , condensed milk and serum.

The unique features of the kit are:

1. High recovery (80-115%), rapid (10-40 minutes), and cost-effective extraction methods.
2. High sensitivity (0.05 ng/g or ppb) and low detection limit (0.025 ng/g or ppb) for shrimp, fish and meat samples.
3. High reproducibility.
4. A quick ELISA assay (less than 1 hour regardless of number of samples).

Principles of Testing

The kit is based on a competitive colorimetric ELISA assay. The drug of interest has been coated in the plate wells. During the analysis, sample and the CAP antibody are added along with secondary antibody, tagged with a peroxidase enzyme. If the CAP residue is present in the sample, it will compete for the CAP antibody, thereby preventing the antibody from binding to the CAP attached to the well. The resulting color intensity, after addition of the HRP substrate (TMB), has an inverse relationship with the CAP residue concentration in the sample.

Reagents And Materials Provided

Chloramphenicol (CAP) ELISA Test Kit has the capacity for 96 determinations or testing of 42 samples in duplicate (assuming 12 wells for standards). Return any unused microwells to the foil bag and reseal them with the desiccant provided in the original package.

1. CAP Coated Plate; 1×96-well Plate (8 wells × 12 strips); store at 2-8°C
2. CAP Standards:
   a. Negative control (white cap tube); 1.0 mL; store at 2-8°C
   b. 0.05 ng/mL (yellow cap tube); 1.0 mL; store at 2-8°C
   c. 0.15 ng/mL (orange cap tube); 1.0 mL; store at 2-8°C
   d. 0.5 ng/mL (pink cap tube); 1.0 mL; store at 2-8°C
   e. 1.5 ng/mL (purple cap tube); 1.0 mL; store at 2-8°C
   f. 4.5 ng/mL (blue cap tube); 1.0 mL; store at 2-8°C
   g. 10 ng/mL (spiking, optional, red cap tube); 1.0 mL; store at 2-8°C
3. Antibody#1; 6 mL; store at 2-8°C
4. HRP-Conjugated Antibody#2; 6 mL; store at 2-8°C
5. 10× Sample Extraction Buffer**; 25 mL; store at 2-8°C

6. 20× Wash Solution; 30 mL; store at 2-8°C
7. Stop Buffer; 12 mL; store at 2-8°C
8. TMB Substrate; 12 mL; store at 2-8°C

Note: If you are not planning to use the kit for over 1 month, store Standard Stock, Antibody#1 and HRP-Conjugated Antibody#2 at -20°C or in a freezer.

Materials Required But Not Supplied

1. Microtiter plate reader (450 nm)
2. Incubator
3. Tissue Mixer (e.g. Omni Tissue Master Homogenizer)
4. Rotary evaporator or nitrogen gas
5. Vortex mixer (e.g. Gneie Vortex mixer from VWR)
6. 10, 20, 100 and 1000 µL pipettes
7. Multi-channel pipette: 50-300 µL (Optional)
8. Ethyl acetate
9. n-Hexane

Storage

Store the kit at 2-8°C. The shelf life is 12 months when the kit is properly stored.

Specimen Collection And Preparation

Be sure samples are properly stored. In general, samples should be refrigerated at 2-4°C for no more than 1-2 days. Freeze samples to a minimum of -20°C if they need to be stored for a longer period. Frozen samples can be thawed at room temps (20-25°C / 68-77°F) or in a refrigerator before use.

Preparation of 1×Sample Extraction Buffer:

Mix 1 volume of 10× Sample Extraction Buffer with 9 volumes of distilled water.

1. Fish/Shrimp/Meat (Beef/Chicken/Pork)/Egg.

Method A:

1) Homogenize a reasonable amount of sample with a suitable mixer.
2) Weight out 3g of the homogenized sample and mix with 6 mL of ethyl acetate, vortex 3 min at max speed.
3) Centrifuge for 5 minutes at 4,000 x g at room temperature (20-25°C / 68-77°F).
4) Transfer 4 mL of the ethyl acetate supernatant (corresponding to 2g of the original sample) into a new vial and use a rotary evaporator to dry the sample in a 60-70°C water bath under reduced pressure. Alternatively, the sample can be dried by blowing nitrogen gas in a 60-70°C water bath.
5) Dissolve the dried residue in 2 mL of n-hexane.
6) Add 1 mL of 1×Sample Extraction Buffer and mix by vortexing at maximum speed for 2 minutes.
7) Centrifuge for 10 minutes at 4,000 x g at room temp. Discard the upper hexane layer.

8) Use 50µL of the lower aqueous layer per well for the assay. In case emulsion happened, remove the upper n-hexane layer and incubate the lower aqueous layer in water bath for 3 min at 80-95°C.

Note: Dilution factor: 0.5.

Method B:

1) Mix 1 g of the homogenized sample with 0.5 mL of 1×Sample Extraction Buffer, 3.5 mL of distilled water, 0.5 mL of 1 M HCl and 20µL of 50 mM 2-Nitrobenzaldehyde by vortexing for 30 seconds.
2) Incubate at 50°C-55°C for 3 hours. Vortex the sample for 5 seconds every hour during the incubation.
3) Add 5 mL of 0.1 M K_2HPO_4, 0.4 mL of 1 M NaOH and 6 mL of ethyl acetate, vortex for 5 minutes.
4) Centrifuge at 4,000 x g for 10 minutes at room temperature (20-25°C).
5) Transfer 3 mL of the ethyl acetate supernatant (corresponding to 0.5 g of the original sample) into a new vial (Avoid the lower aqueous layer! If contaminated with lower layer, centrifuge the extracted ethyl acetate for 5 minutes at 4,000 x g again and get the upper organic layer).In case emulsion happened and the upper ethyl acetate layer was less than 3 mL,incubate the sample in water bath for 3 minutes at 85°C. Use a rotary evaporator to dry the sample in a 60-70°C water bath under reduced pressure. Alternatively,the sample can be dried by blowing nitrogen gas in a 60-70°C water bath.
6) Dissolve the dried residue in 1 mL of n-hexane (or n-heptane).
7) Add 1 mL of 1× Sample Extraction Buffer, vortex the sample for 2 minutes.
8) Centrifuge the sample at 4,000 x g for 10 minutes at room temperature (20-25°C / 68-77°F).
9) Use 50µL of the lower aqueous layer per well for the assay.

Note: Dilution factor: 2.

2. Honey

1) Dissolve 2 g of the honey sample with 4 mL of distilled water into a centrifugal glass vial.
2) Add 4 mL of ethyl acetate, vortex for 3 minutes at maximum speed.
3) Centrifuge for 5 minutes at 4,000 x g at room temperature (20-25°C / 68-77°F).
4) Transfer 1 mL of the ethyl acetate supernatant (corresponding to 0.5 g of the original sample) into a new vial and use a rotary evaporator to dry the sample in a 60-70°C water bath under reduced pressure. Alternatively, the sample can be dried by blowing nitrogen gas in a 60-70°C water bath.
5) Dissolve the dried residue in 2 mL of n-hexane.
6) Add 0.5 mL of 1× Sample Extraction Buffer and mix by vortexing at maximum speed for 2 minutes.
7) Centrifuge for 10 minutes at 4,000 x g at room temp. Discard the upper hexane layer.
8) Use 50µL of the lower aqueous layer per well for the assay. In case emulsion happened, remove the upper n-hexane layer and incubate the lower aqueous layer in water bath for 3 min at 80-95°C.

Note: Dilution factor: 1.

3. Condensed Milk

1) Weight out 1 g of the condensed milk sample in a centrifugal glass vial, add 1 mL of distilled water,vortex well and add 6 mL of ethyl acetate, vortex for 3 minutes at maximum speed.
2) Centrifuge for 5 minutes at 4,000 x g at room temperature (20-25°C / 68-77°F).
3) Transfer 3 mL of the ethyl acetate supernatant (corresponding to 0.5 g of the original milk powder sample) into a new vial and use a rotary evaporator to dry the sample in a 60-70°C water bath under reduced

pressure. Alternatively, the sample can be dried by blowing nitrogen gas in a 60-70°C water bath.

4) Dissolve the dried residue in 2 mL of n-hexane.
5) Add 0.5 mL of 1× Sample Extraction Buffer and mix by vortexing at maximum speed for 2 minutes.
6) Centrifuge for 10 minutes at 4,000 x g at room temp. Discard the upper hexane layer.
7) Use 50µL of the lower aqueous layer per well for the assay. In case emulsion happened, remove the upper n-hexane layer and incubate the lower aqueous layer in water bath for 3 min at 80-95°C.

Note: Dilution factor: 1.

4. Milk

1) Weight out 1 g of the milk sample in a centrifugal glass vial, add 5 mL of ethyl acetate, vortex for 3 minutes at maximum speed.
2) Centrifuge for 5 minutes at 4,000 x g at room temperature (20-25°C / 68-77°F).
3) Transfer 2.5 mL of the ethyl acetate supernatant (corresponding to 0.5 g of the original milk powder sample) into a new vial and use a rotary evaporator to dry the sample in a 60-70°C water bath under reduced pressure. Alternatively, the sample can be dried by blowing nitrogen gas in a 60-70°C water bath.
4) Dissolve the dried residue in 2 mL of n-hexane.
5) Add 0.5 mL of 1× Sample Extraction Buffer and mix by vortexing at maximum speed for 2 minutes.
6) Centrifuge for 10 minutes at 4,000 x g at room temp. Discard the upper hexane layer.
7) Use 50µL of the lower aqueous layer per well for the assay. In case emulsion happened, remove the upper n-hexane layer and incubate the lower aqueous layer in water bath for 3 min at 80-95°C.

Note: Dilution factor: 1

5. Milk Powder

1) Weight out 2 g of milk powder in a centrifugal glass vial, add 10 mL of distilled water and dissolve by shaking.
2) Centrifuge at 4,000 x g for 5 minutes, discard the upper lipid layer.
3) Take 4 mL of milk from the aqueous layer, and add 6 mL of ethyl acetate, vortex for 3 minutes at maximum speed.
4) Centrifuge for 5 minutes at 4,000 x g at room temperature (20-25°C / 68-77°F).
5) Transfer 3 mL of the ethyl acetate supernatant (corresponding to 0.4 g of the original milk powder sample) into a new vial and use a rotary evaporator to dry the sample in a 60-70°C water bath under reduced pressure. Alternatively, the sample can be dried by blowing nitrogen gas in a 60-70°C water bath.
6) Dissolve the dried residue in 800µL 1×Sample Extraction Buffer
7) Use 50 µL per well for the assay.

Note: Dilution factor: 2. For this preparation, we recommend using standard 0.5 ng/g or ppb as the cut off value for positive samples since negative samples could show considerable background effects (in some cases between standards 0.05 and 0.15 ng/g).

6. Cell Lysis Solution/Serum

1) Centrifuge 0.5 mL of sample at 2,000 x g for 5 minutes. Recover the supernatant and dilute it 5 fold with 1× Sample Extraction Buffer (For example, add 100µL sample to 400µL 1×Sample Extraction Buffer).
2) Use 50 µL per well for the assay.

Note: Dilution factor: 5.

Reagent Preparation

IMPORTANT: All reagents should be brought up to room temperature before use (1-2 hours at 20-25°C / 68-77°F); Make sure you read "Precautions" section. Solutions should be prepared just prior to ELISA test. All reagents should be mixed by gently inverting or swirling prior to use. Prepare volumes that are needed for the number of wells being run. Do not return the reagents to the original stock tubes/bottles. Using disposable reservoirs when handling reagents can minimize the risk of contamination and is recommended.

Preparation of 1× Wash Solution

Mix 1 volume of the 20× Wash Solution with 19 volumes of distilled water.

Assay Procedure

Label the individual strips that will be used and aliquot reagents as the following example:

Component	Volume per Reaction	24 Reactions
Antibody #1	50 µL	1.2 mL
HRP Conjugate	50 µL	1.2 mL
1X Wash Solution	1.0 mL	24 mL
Stop Buffer	100 µL	2.4 mL
TMB Substrate	100 µL	2.4 mL

1. Add 50 µL of each CAP Standards in duplicate into different wells (Add standards to plate only in the order from low concentration to high concentration).
2. Add 50 µL of each sample in duplicate into different sample wells.
3. Add 50 µL of HRP-Conjugated Antibody #2 and 50 µL of Antibody #1 ,then mix the well by gently rocking the plate manually.
4. Incubate the plate for 30 minutes at room temperature (25°C /77°F).
5. Wash plate 4 times with 250 µL of 1x Wash Solution. After the last wash, invert the plate and gently tap the plate dry on paper towels (Perform the next step immediately after plate washings. Do not allow the plate to dry between working steps).
6. Add 100 µL of TMB substrate. Time the reaction immediately after adding the substrate. Mix the solution by gently rocking the plate manually for 1 minute while incubating (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).
7. After incubating for 15 minutes at room temperature (25°C /77°F), add 100 µL of Stop Buffer to stop the enzyme reaction.
8. Read the plate as soon as possible following the addition of Stop Buffer on a plate reader with 450 nm wavelength (Before reading, use a lint-free wipe on the bottom of the plate to ensure no moisture or

fingerprints interfere with the readings).

Calculation

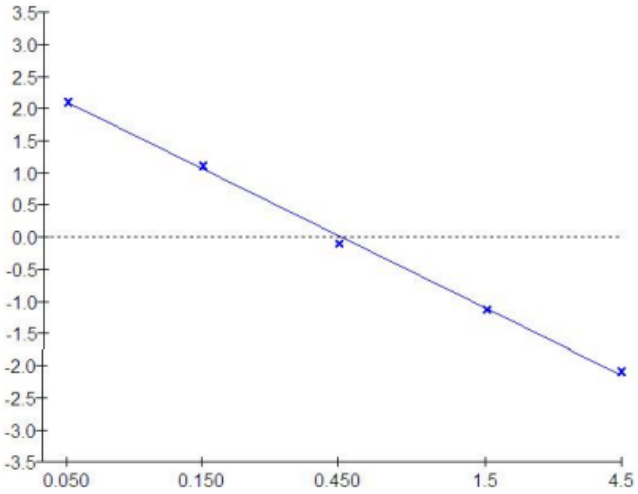
A standard curve can be constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ng/mL on a logarithmic curve.

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

Use the mean relative absorbance values for each sample to determine the corresponding concentration of the tested drug in ng/mL from the standard curve. A special program with Excel functionality.

Typical Standard Curve

The following figure is a typical chloramphenicol standard curve.



Sensitivity

Sample Type	**Detection Limit** (ng/g or ppb)
Fish/Shrimp/Meat/ Egg	0.025/0.1
Honey	0.05
Condensed Milk	0.05
Milk Powder	0.1
Milk	0.05
Cell Lysis Solution/Serum	0.25

Specificity

Analytes	Cross-Reactivity (%)
Chloramphenicol (CAP)	100.0
Chloramphenicol Glucuronide	76.8
Chloramphenicol base	<0.5
Thiamphenicol	< 0.05
Tetracyclines	< 0.01
Gentamicin	< 0.01
Ampicillin	< 0.01
Florfenicol	< 0.01

Precautions

1. The standards contain chloramphenicol. Handle with particular care.
2. Do not use the kit past the expiration date.
3. Do not intermix reagents from different kits or lots. CAP-HRP CONJUGATES AND PLATES ARE KIT-AND LOT-SPECIFIC.
4. Try to maintain a laboratory temperature of 20°–25°C (68°–77°F). Avoid running assays under or near air vents, as this may cause excessive cooling, heating and/or evaporation. Also, do not run assays in direct sunlight, as this may cause excessive heat and evaporation. Cold bench tops should be avoided by placing several layers of paper towel or some other insulation material under the assay plates during incubation.
5. Make sure you are using only distilled or deionized water since water quality is very important.
6. When pipetting samples or reagents into an empty microtiter plate, place the pipette tips in the lower corner of the well, making contact with the plastic.
7. Incubations of assay plates should be timed as precisely as possible. Be consistent when adding standards to the assay plate. Add your standards first and then your samples.
8. Add standards to plate only in the order from low concentration to high concentration as this will minimize the risk of compromising the standard curve.
9. Always refrigerate plates in sealed bags with a desiccant to maintain stability. Prevent condensation from forming on plates by allowing them equilibrate to room temperature 20°–25°C (68°–77°F) while in the packaging.