



Florfenicol ELISA Kit

11/19

(Catalog # E4780-100; 96 assays, Storage at 4°C)

I. Introduction:

Florfenicol is a broad-spectrum, primarily bacteriostatic, antibiotic with a range of activity similar to that of chloramphenicol, including many gram-negative and gram-positive organisms. It has a same mechanism of action as chloramphenicol i.e. inhibition of protein synthesis. Signs of Florfenicol toxicity are varied and include diarrhea and hyperbilirubinemia in horses, diarrhea and decreased feed consumption and rumen activity in cattle. Overdose or prolonged florfenicol administration can cause fatal bone marrow suppression. Florfenicol ELISA Kit is based on Competitive ELISA principle. The micro-plate provided in this kit has been pre-coated with Florfenicol. During the reaction, Florfenicol in the samples or standard competes with Florfenicol coated on the plate for binding to the anti-Florfenicol antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and TMB substrate is for color development. There is a negative correlation between the OD value of samples and the concentration of Florfenicol. The concentration of Florfenicol in the samples can be calculated by comparing the OD of the samples to the standard curve.

II. Applications:

In vitro, quantitative determination of Florfenicol **Sensitivity:** 0.15 ppb (ng/mL) **Detection Range:** Tissue, Liver, Honey, Milk - 0.075ppb; Feed, Milk powder - 0.15 ppb **Sample recovery rate:** Tissue, Liver - 85%±20%; Honey- 85%±25%; Feed, Milk - 75%±25%. **Cross-reactivity:** Florfenicol -100%; Thiamphenicol - 0.1%

III. Sample Type:

Tissue, Honey

IV. Kit Contents:

Components	E4780-100	Part Number
Micro ELISA Plate	96 wells	E4780-100-1
Standard	6 X 1 ml	E4780-100-2
HRP Conjugate	5.5 ml	E4780-100-3
Antibody Working Solution	5.5 ml	E4780-100-4
Substrate Reagent A	6 ml	E4780-100-5
Substrate Reagent B	6 ml	E4780-100-6
Stop Solution	6 ml	E4780-100-7
Wash Buffer (20X)	40 ml	E4780-100-8
Reconstitution Buffer (2X)	50 ml	E4780-100-9
Plate Sealer	3	E4780-100-10

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Ethyl acetate, N-hexane, Acetonitrile, Na₂Fe(CN)₅(NO)•2H₂O, ZnSO₄·7H₂O
- Clean Eppendorf tubes for preparing standards or sample dilutions

VI. Storage and Handling:

Store at 4°C.

VII. Reagent and Sample Preparation:

Bring all reagents to room temperature before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

- Wash Buffer (20X): Dilute 20X Concentrated Wash Buffer to 1X with deionized water.
- Reconstitution Buffer (2X): Dilute 2X Reconstitution Buffer with deionized water. Mix 2x Reconstitution Buffer (V): Deionized water (V) =1:1). The Reconstitution buffer can be store at 4°C for a month.
- 0.36M Na₂Fe (CN)₅(NO)•2H₂O Solution: Dissolve 10.7 g of Na₂Fe(CN)₅(NO)•2H₂O to 100 mL with deionized water.
- 1.04M ZnSO₄·7H₂O Solution: Dissolve 29.8 g of ZnSO₄·7H₂O to 100 mL with deionized water.
- Acetonitrile -Water Solution: Acetonitrile (V): Deionized water (V) =84:16
- Standard:

Standard	S1	S2	S3	S4	S5	S6
Concentration (ppb)	0	0.15	0.45	1.35	4.05	12.15





VIII. Sample Preparation:

Sample pretreatment:

Pretreatment of tissue (fish, shrimp, livestock), liver sample:

Homogenize tissue, liver samples with homogenizer. Weigh accurately 3 ± 0.05 g of homogenate sample into the 50 mL centrifuge tube, add 6 mL of Ethyl acetate. Shake well for 5 min, centrifuge at 4000 r/min for 10 min at room temperature. Take 4 mL of supernatant to another tube, dry at 50-60°C with nitrogen evaporators or water bath. Dissolve the residual with 1 mL N-hexane, add 1 mL of Reconstitution Buffer and mix well for 1 min. Centrifuge at 4000 r/min at room temperature for 10 min. Discard the upper n-hexane, take 50 µl lower liquid for analysis.

Note: Sample dilution factor: 0.5, minimum detection limit: 0.075 ppb.

Pretreatment of honey sample:

Weigh 2 ± 0.05 g of honey into a centrifuge tube; add 4 ml of Ethyl acetate and 4 mL of deionized water. Oscillate for 2 min, centrifuge at 4000 r/min for 10 min at room temperature. Take 2 ml of supernatant to another tube, dry at 50-60°C with nitrogen evaporators or water bath. Dissolve the residual with 0.5 ml of Reconstitution Buffer. Take 50 µl sample for analysis. Note: Sample dilution factor: 0.5, minimum detection limit: 0.075 ppb.

• Pretreatment of milk sample:

Centrifuge the milk at 4000 r/min for 10 min at 15°C, discard upper fat layer. Take 5 ml of fat free milk into 50 ml centrifuge tube, add 250 μ l of 0.36M Na₂Fe (CN)₅(NO)•2H₂O Solution and mix well for 30s, then add 250 μ l of 1.04M ZnSO₄•7H₂O Solution and mix well for 30s, centrifuge at 4000 r/min for 10 min at 15°C. Take 2.2 mL of the supernatant to another centrifuge tube, add 4 ml of Ethyl acetate and oscillate for 2 min, centrifuge at 4000 r/min for 10 min at room temperature. Take 2 mL of supernatant to another centrifuge tube, dry at 50-60°C with nitrogen evaporators or water bath. Dissolved the residue with 0.5 mL of Reconstitution Buffer, mix well. Take 50 μ l for detection and analysis.

Note: Sample dilution factor: 0.5, minimum detection limit: 0.075 ppb.

• Pretreatment of milk powder sample:

Weigh 2 ± 0.05 g milk powder into centrifuge tube, dissolved with 10 mL deionized water, add 1 ml of $0.36 \text{ Na}_2\text{Fe}(\text{CN})_5(\text{NO})\cdot 2\text{H}_2\text{O}$ Solution and 1mL of 1.04M ZnSO₄•7H₂O Solution. Shake well for 2 min and centrifuge at 4000 r/min for 10 min at 15°C. Take 3.6 ml of the supernatant to another centrifuge tube, add 6 mL of Ethyl acetate and shake well for 5 min, centrifuge at 4000 r/min for 10 min at room temperature. Take 4 mL of supernatant to another centrifuge tube, dry at 50-60°C with nitrogen evaporators or water bath. Dissolve the residue with 0.4 mL of Reconstitution Buffer, mix well. Take 50 µl for detection and analysis. **Note: Sample dilution factor: 1, minimum detection limit: 0.15 ppb**

IX. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

- 1. Add 50 µl of each standard or samples into appropriate wells.
- 2. Add 50 μl of HRP Conjugate to each well. Add 50 μl of Antibody Working Solution. Cover the plate with the sealer provided in the kit. Gently mix and incubate for 30 min. at 25°C.
- **3.** Aspirate the solution from each well add 300 μl of **1x wash buffer** to each well. Leave it for 30 sec, aspirate the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 5 times.
- 4. Add 50 μl of Substrate Reagent A to each well and then add 50 μl of Substrate Reagent B. Cover with a plate sealer. Incubate for about 15 min at 25°C. Protect the plate from light.
 - Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min.
- 5. Add 50 µl of Stop Solution to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
- 6. Read the absorbance in micro plate reader set to 450 nm reference wavelength 630 nm. This step should be performed within 10 min after stop reaction.

X. Calculation:

Create a standard curve by plotting the absorbance percentage of each standard on the yaxis against the log concentration on the x-axis to draw a semi logarithmic plot. Add average absorbance value of sample to standard curve to get corresponding concentration. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.

Absorbance (%)=A/A₀ ×100%

A: Average absorbance of standard or sample

A₀ : Average absorbance of 0 ppb Standard







Concentration of standard (ppb)	OD-1	OD-2	Average OD
0	2.2123	2.2324	2.2224
0.15	1.7997	1.7056	1.7527
0.45	1.3605	1.3182	1.3394
1.35	0.8475	0.8566	0.8521
4.05	0.4091	0.4016	0.4054
12.15	0.2153	0.2037	0.2095

Typical standard curve and data is provided below for reference only. A standard curve must be run with each assay

XI. RELATED PRODUCTS:

- Cimaterol ELISA Kit (E4771) ٠
- Norfloxacin ELISA Kit (E4776) ٠
- Diazepam ELISA Kit (E4772) Tylosin ELISA Kit (E4779) •
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FOR RESEARCH USE ONLY! Not to be used on humans.