# Florfenicol ELISA Kit

Product #: E6007-96T



**Manufacturer Information**

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**1. Description**

Florfenicol, is a new veterinary special chloramphenicol class of broad-spectrum antibacterial drugs successfully developed in the late 1980s, white or white crystalline powder, no odor, bitter taste, long-term or repeated large intake of Florfenicol may be harmful to human health.
This ELISA Kit is based on indirect competitive ELISA to detect florfenicol in sample within 75 minutes.

**2. Principle**

This ELISA Kit is based on indirect competitive ELISA, the coupled antigen was pre-coated in the microwells, the florfenicol will compete the antibody with the pre-coated antigen, then add enzyme conjugate and TMB substrate successively, the OD value of the sample is negatively correlated with the content of florfenicol contained in the sample. The standard curve is fitted by regression and multiplied by the corresponding dilution ratio to obtain the content of florfenicol in the sample.

**3. Application**

This kit is applicable for quantitative determination of florfenicol in milk and milk powder.

**4. Instrument and material required**

1) ELISA reader, with 450/630nm
2) Balance, 0.0001g
3) Centrifuge tube, 2mL, 15mL, 50mL
4) Vortex mixer
5) Micropipette, 20-200μL, 100-1000μL
6) Multi-channel pipette, 250μL

**5. Reagent required**

Deionized water

**6. Kit components**

1) Microtiter plate, 96wells, 1 plate
2) Florfenicol standards, 1mL/vial, 7 vials, 0, 0.025, 0.075, 0.225, 0.675, 2.025ng/mL, 100ng/mL
3) Enzyme conjugate, 12mL, with red cap
4) Antibody Solution, 7mL, with green cap
5) Foundation liquid 10mL, with transparent cap
6) TMB Substrate, 12mL, with brown cap
7) Stop solution 12mL, with white cap
8) 20x Wash buffer, 50mL, with transparent cap
9) manual, 1 set

**7. Buffer preparation**

**Wash buffer**: Dilute 20x wash buffer with deionized water, in the volume ratio of 1:19, for example, 10mL 20x wash buffer + 190mL deionized water, mix thoroughly. This diluted wash buffer can be stored at 4°C for 1 month.

**8. Notice and precautions before assay**

1) Make sure the ELISA kit and all reagents are returned to room temperature (20-25 °C). For example, keep these reagent and kits at room temperature for at least 30min.
2) Return unused kit components to 2-8 °C.
3) Washing step is important for the reproducibility of the kit, please follow this instruction carefully.

4) Cover the ELISA plate during all incubation. Avoid direct sunlight.

**9. Sample preparation**

**Precautions before prepare samples:**

1) Use disposable tips during the test. Change new tip for different sample / reagent.
2) Make sure all lab wares are clean and ready to use.
3) Prepared sample shall be analyzed immediately after dilution.

**10. Sample handling**

**10.1 Milk**

Take 50μL milk sample for assay.

**10.2 Milk or goat powder**

Take 1.0±0.005g sample into a new centrifuge tube, add 8mL deionized water, vortex for 3min to dissolve, and then take 50μL sample for assay.

**11. Assay procedures**

1) Return the ELISA kit and all reagents to room temperature (20-25 °C). For example, keep these reagent and kits at room temperature for at least 30min.
2) Take needed microwells and zip rest in the zip-bag and return to 2-8 °C.
3) Layout the plate and record sample and standard well positions. It is recommended to run all tests in duplicates.
4) Add sample buffer into each well, 50μL /well.
5) **Add sample/standard/antibody**: add sample/standard into the wells, 50μL per each, then add antibody solution, 50μL per well, shake gently and then cover the plate and incubate at **25 °C for 30min**.
6) **Wash**: take out the plate and pour the liquid out. Use the diluted wash buffer to wash the plate, 250μL/well. Wash for 4-5 times with interval of 10s. The pour the liquid out and tap the plate against absorbent paper. Eliminate the air bubble in the wells with micropipette tip if the bubble exists.
7) **Add enzyme conjugate**: add enzyme conjugate, 100μL per well, shake gently and then incubate at **25°C for 30min**. Then take out and repeat **Wash Step**.
8) **Coloration**: add TMB substrate, 100μL per well, and then cover the plate and incubate **25 °C for 15min**.
9) **Stop the reaction**: add stop solution, 100μL per well, shake gently and read the plate with ELISA reader at 450nm. Read the plate within 5min after adding stop solution.

**12. Result Calculation**

This kit is based on competitive ELISA, thus the OD values is inversely proportional to the florfenicol content contained in sample.

**12.1 Quantitative analysis**

Calculation of absorbance: the percentage absorbance of standard product or sample is equal to the average absorbance value of standard product or sample (double hole) divided by the average absorbance value of blank standard product, and then multiplied by 100%, i.e

$$\text{percentage absorbance } (\%) = \frac{\text{B}}{B_0} \times 100\%$$

B - the average absorbance value of standard product or sample

$B_0$- the average absorbance value of blank standard product

**12.2 Standard curve**

The standard curve was drawn by taking the percentage absorption of standard substance as the vertical coordinate and the logarithm of concentration of standard substance florfenicol as the horizontal coordinate. Put the percentage absorption rate of samples into the standard curve, read the corresponding concentration of samples from the standard curve, and multiply the corresponding dilution times to obtain the actual concentration of florfenicol in samples.

**13. Sample dilution factor:**

Milk: 1 time

Milk power: 1 time

**14. LOD of sample**

Milk: 0.025ng/mL

Milk power: 0.025ng/mL

**15. Specifications of the kit**

1) Sensitivity: 0.025ng/mL
2) Specificity: 100% to florfenicol, no cross reaction with chloramphenicol and thiamphenicol.
3) Recovery: 70% - 120%
4) Precision: C.V<10%.

**16. Cautions and tips for the test**

1) Lower room temperature, e.g., lower than 20 ◦C may cause lower OD values. Please make sure all reagent and kit components are returned to room temperature.
2) Wash step is vital for the reproducibility of the kit. Please wash according to the kit instruction. Do not let the plate dry during wash. Continue the next operations immediately after wash step.
3) Shake each reagent gently before use.
4) Stop solution is acidic, please handle with care.
5) Do not use expired kits and reagents. Do not mix the reagent and kits from different LOT.
6) The kit is stored at 2-8◦C(36-46◦F), do not freeze.
7) TMB substrate is sensitive to sunlight. Avoid direct sunlight.
8) If Standard 1 (0ng/L) OD is lower than 0.5, please do not use. The kit may be expired or deteriorated.
9) The coloration step takes 15min. You can prolong it to 20min-25min if the color of the well is too light. On the contrary, please reduce the incubation time.
10) The incubation is 25◦C /98.6 ◦F, lower or higher temperature will cause changes of OD and sensitivity of the kits, which may affect the result of the assay.

**14. Storage and expiration**

The kit is valid for 12months when stored at 2-8 ◦C. LOT and Expiry information are printed on the package.