VERSION 1.01

FLORFENICOL ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Florfenicol ELISA Test Kit

Catalogue Number. IP100008

Principle

This test kit is based on the competitive enzyme immunoassay for the detection of Florfenicol in samples. The coupling antigens are pre-coated on the micro-well stripes. The Florfenicol in the sample and the conjugate antigens pre-coated on the micro-well stripes compete for the anti-Florfenicol antibodies. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value has a negative correlation with the Florfenicol concentration in the sample. This value is compared to the standard curve and the Florfenicol concentration is subsequently obtained.

Technical specifications

Sensitivity: 0.1ppb
Incubator temperature: 25℃
Incubator time: 30min～15min
Detection limit: Honey, tissue 0.1ppb, Milk 0.2ppb
Recovery rate: Tissue, honey, milk 90 ±30%
Cross-reaction rate: Florfenicol 100%, Florfenicol Amine 11%, Thiamphenicol < 0.1%, Chloramphenicol < 0.1%

Components

1	Micro-well strips	12 strips with 8 removable wells each	
2	6× standard solution (1mL each)	0ppb, 0.1ppb, 0.2ppb, 0.4ppb, 0.8ppb, 1.6ppb	
3	Enzyme conjugate	7ml	red cap
4	Antibody working solution	7ml	blue cap
5	Substrate A	7ml	white cap
6	Substrate B	7ml	black cap
7	Stop solution	7ml	yellow cap
8	20× concentrated washing buffer	40ml	white cap
9	2×concentrated redissolving solution	50ml	transparent cap

Materials required but not provided

Equipments: microplate reader, homogenizer, printer, nitrogen-drying device, oscillator, centrifuge, measuring pipets, balance(a reciprocal sensibility of 0.01 g), incubator.

Micropipettors: single-channel 20-200μL and 100-1000 μL, and multi-channel 30 ~ 300 μl;

Reagents: Acetonitrile, ethyl acetate, N-hexane

Sample pre-treatment

Instructions

The following points must be dealt with before the pre-treatment of any kind of sample:

1) Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents.
2) Before the experiment, each experimental utensil must be clean and should be re-cleaned if necessary, in order to avoid the contamination that interferes with the experimental results.

Solution preparation before sample pre-treatment

Solution I Sample redissolving solution:
Dilute the 2× concentrated redissolving solution with deionized water at 1:1 (1 part 2× concentrated redissolving solution +1 part deionized water).
Solution Ⅱ Sample extracting solution:
Mix Acetonitrile and Ethyl acetate at 1:2 evenly (1 part Acetonitrile + 2 parts Ethyl acetate)

Samples preparation

a) Tissue (Chicken/liver, pork/liver, shrimp, fish etc.)

1. Weight 3.0 ± 0.05 g of the homogenized sample into a 50ml centrifuge tube, first add 3ml deionized water to mix evenly, then add 6 mL of ethyl acetate, shake properly for 2 min, centrifuge at above 4000 r/min at room temperature for 10 min.
2. Take 2 mL of the supernatant, blow to dry with nitrogen at 50-60 ℃.
3. Add 2 mL N-hexane to dissolve the dry residues, then add in 1 mL of the sample redissolving solution, mix for 30 seconds; centrifuge at above 4000 r/min at room temperature for 5 min. Remove up-layer organic phase.
4. Take 50 µL of the down-layer for analysis.

Fold of dilution of the sample: 1

b) Honey

1. Put 2±0.05 g honey sample into centrifugal tube, dissolved with 4 mL deionized water. Add 4 mL ethyl acetate, shake for 2 min. Centrifuge at above 4000 r/min at room temperature for 10 min.
2. Transfer 2ml supernatant into another centrifuge tube, blow to dry with nitrogen at 50-60 ℃. Add 1 mL of the sample redissolving solution to dissolve dry residue, mix for 30 seconds.
3. Take 50 µL for analysis.

Fold of dilution of the sample: 1

c) Milk

1. Take 1mL of homogenized milk sample into a 5ml centrifuge tube, add 2ml Sample extracting solution, shake for 1min, centrifuge at above 4000 r/min at room temperature for 10 min.
2. Take 1 mL of the supernatant, blow to dry with nitrogen at 50-60 ℃.
3. Add 1 mL N-hexane to dissolve the dry residues, then add 1 mL of the sample redissolving solution, mix for 30 seconds; centrifuge at above 4000 r/min at room temperature for 5 min. Remove up-layer organic phase.
4. Take 50 µL of the down-layer for analysis.

Fold of dilution of the sample: 2

ELISA procedures

Instructions

1) Bring all reagents and micro-well strips to the room temperature before use (20-25 ℃).
2) Return all reagents to 2-8 ℃ immediately after use.

3) The reproducibility of the ELISA analysis, to a large degree, depends on the consistency of plate washing. The correct operation of plate washing is the key point in the ELISA . procedures.
4) For the incubation at constant temperatures, all the samples and reagents must avoid light exposure, and each microplate should be sealed by the cover membrane.

Operation procedures

1. Bring all reagents and micro-well strips to the room temperature before use (20-25 ℃).
2. Return all reagents to 2-8 ℃ immediately after use.
3. The reproducibility of the ELISA analysis, to a large degree, depends on the consistency of plate washing. The correct operation of plate washing is the key point in the ELISA . procedures.
4. For the incubation at constant temperatures, all the samples and reagents must avoid light exposure, and each microplate should be sealed by the cover membrane.

Test implementation

1 Take out all the necessary reagents from 2~8 ℃ environment, bring them to the room temperature (20-25 ℃) for at least 30 min, note that each liquid reagent must be shaken evenly before use.
2. Take the required micro-well strips and plate frames. Re-sealed the unused microplate, store at 2-8℃, not frozen.
3. Solution preparation: dissolve 40ml of the 20×concentrated washing buffer with deionized water at 1:19 (1 part of 20×concentrated washing buffer + 19 parts of deinonized water) or just to the required volume for use.
4. Numbering: number the micro-wells according to samples and standard solution; each testing sample and standard solution should be performed in duplicate; record their positions.
5. Add 50 µL of the sample or standard solution to separate duplicate wells, add 50 µL of the enzyme conjugate, then add 50 µL of the antibody working solution into each well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, and incubate at 25 ℃ for 30 min.
6. Wash the microplate with the washing buffer at 250 µL/well for 4-5 times. Each time soak the well with the washing buffer for 15-30s and then flap to dry (if there are the bubbles after flapping, cut them with the clean tips).
7. Coloration: add 50 µL of the substrate A solution and then 50 µL of the substrate B solution into each well. Mix gently by shaking the plate manually, and incubate at 25 ℃ for 15 min at dark for coloration.
8. Determination: add 50 µL of the stop solution into each well, Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450 nm to determine the OD value (we recommend to read the OD value at the dual-wavelength 450/630 nm within 5 min).

Result judgment

There are two methods to judge the results; the first one is the rough judgment, while the second is the quantitative determination. Note that the OD value of the sample has a negative correlation with the content of Florfenicol.

Qualitative determination

The concentration range (ng/mL) can be obtained from comparing the average OD value of the sample with that of the standard solution. Assuming that the OD value of the sample Ⅰ is 0.3, and that of the sample Ⅱ is 1.0, the OD value of standard solutions is: 2.243 for 0ppb, 1.816 for 0.1ppb, 1.415 for 0.2ppb, 0.74 for 0.4ppb, 0.313 for 0.8ppb, 0.155 for 1.6ppb, accordingly the concentration range of the sample Ⅰ is 0.8 to 1.6, and that of the sample Ⅱ is 0.2 to 0.4ppb.

Quantitative determination

The mean values of the absorbance values obtained for the average OD value (B) of the sample and the standard solution divided by the OD value (B_0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is,

Percentage of absorbance value = (B / B_0) × 100%

B—the average OD value of the sample or the standard solution

B_0—the average OD value of the 0 ng/mL standard solution

Draw the standard curve with the absorption percentages of the standard solutions and the semilogarithm values of the Florfenicol standard solutions (ng/mL) as Y- and X-axis, respectively. Read the corresponding concentration of the sample from the standard curve by incorporating its absorption percentage into the standard curve. The resulting value is subsequently multiplied by the corresponding dilution fold, thus finally obtaining the Florfenicol concentration in the sample.

Using the professional analyzing software of this kit will be more convenient for the accurate and rapid analysis of a large amount of samples. (Please contact us for this software).

Precautions

1. The room temperature below 25 ℃ or the temperature of the reagents and the samples being not returned to the room temperature (20-25 ℃) will lead to a lower standard OD value.
2. Dryness of the microplate in the washing process will be accompanied by the situations including the non-linear standard curves and the undesirable reproducibility; So continue to next step immediately after washing.
3. Mix evenly, otherwise there will be the undesirable reproducibility;
4. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin.
5. Do not use the kit exceeding its expiry date. The use of diluted or adulterated reagents from the kits will lead to the changes in the sensitivity and the detecting OD values. Do not exchange the reagents from the kits of different lot numbers to use.
6. Put the unused microplate into an auto-sealing bag to re-seal it. The standard substance and the colourless color former is light sensitive, and thus they cannot be directly exposed to the light.
7. Discard the colouration solution with any color that indicates the degeneration of this solution. The detecting value of the 0 standard solution of less than 0.5 indicates its degeneration.
8. The optimum reaction temperature is 25 ℃, and too high or too low temperatures will result in the changes in the detecting sensitivity and OD values.

Storage: store at 2-8 ℃, not frozen.
Expiry date: 12 months; date of production is on box.