Florfenicol and Thiamphenicol ELISA KIT DEIA039

1. Background

Florfenicol is a derivative of thiamphenicol, which is broadly applied in animal industry for controlling and treating diseases for it has a relative low cost and strong inhibition against a number of gram bacterium and mycoplasma. The residue of this drug will lead to potential aplastic anemia, so the MRL of which is now restricted to 0.1ppm in food by CAC and many other countries.

This kit is a new product for drug residue detection based on ELISA technology, which can considerably minimize operation errors and work intensity compared with instrumental analysis.

2. Test Principle

This kit is based on indirect-competitive ELISA technology. The microtiter wells are coated with coupling antigen. Florfenicol and thiamphenicol residue in the sample competes with the antigen coated on the microtiter plate for the antibody. After the addition of enzyme labeled anti-antibody, TMB substrate is used to show the color. Absorbance of the sample is negatively related to florfenicol and thiamphenicol residue in it, after comparing with the Standard Curve, multiplied by the dilution factor, florfenicol and thiamphenicol residue quantity in the sample can be calculated.

3. Applications

This kit can be used in quantitative and qualitative analysis of florfenicol and thiamphenicol residue in animal tissue (muscle, liver, fish and shrimp) and feed.

4. Cross-reactions

Florfenicol……100%
Thiamphenicol……..…..……..60%
Florfenicol amine……..……<1%
Chloramphenicol……..……<1%

5. Materials Required

5.1 Equipments

-----Microtiter plate spectrophotometer (450nm/630nm)
-----Rotary evaporator or nitrogen drying instruments
-----Homogenizer
-----Shaker
-----Vortex mixer
-----Centrifuge
-----Analytical balance (inductance: 0.01g)
-----Graduated pipette: 10ml
-----Rubber pipette bulb
-----Volumetric flask: 500ml
-----Glass bottle:15ml
-----Polystyrene Centrifuge tubes:2ml, 50ml
-----Micropipettes: 20μl-200μl, 200μl-1000μl
250μl-multipipette

5.2 Reagents

-----Acetonitrile (AR)
-----N-hexane (AR)
----Trichloroacetic acid (TDA, AR)
-----Deionized water

6. Kit Components

- Microtiter plate with 96 wells coated with antigen
- Standard solutions(6×1ml/bottle)
  0ppb, 0.05ppb,0.15ppb, 0.45ppb,1.35ppb,4.05ppb
- Spiking standard solution: (1ml/bottle) **1ppm**
- Concentrated Enzyme conjugate 1ml…….red cap
- Enzyme conjugate diluent 10ml…….....green cap
- Substrate solution A 7ml……..……...white cap
- Substrate solution B 7ml……..……..……red cap
- Stop solution 7ml……..……yellow cap
- 20×wash solution 40ml……...…transparent cap
- 2×extraction solution 50ml ……blue cap

7. Reagents Preparation

Solution 1: 1% trichloroacetic acid solution

Dissolve 1.0g of trichloroacetic acid with deionized water and to 100ml.

Solution 2: Extraction solution

Dilute the 2Xconcentrate extraction solution with deionized water in the volume ratio of 1:1 (*or according to the requirement*), which will be used for diluting the concentrated antibody solution and

45-16 Ramsey Road Shirley, NY 11967, USA
Tel: 631-624-4882 Fax: 1-631-938-8221
E-mail: info@creative-diagnostics.com
www.creative-diagnostics.com

sample extraction. The diluted extraction solution can be conserved for 1 month at 4℃.

Solution 3: Wash solution

Dilute the 20Xwash solution with deionized water in the volume ratio of 1:19 (*or according to the requirement*), which will be used to rinse the plate. The diluted wash solution can be conserved for 1 month at 4℃.

8. Sample Preparations

8.1 Notice and precautions before operation:

(a) Please use one-off tips in the process of experiment, and change the tips when absorb different reagent.

(b) Make sure that all experimental tools are clean.

(c) Keep untreated samples in freeze.

(d) Treated samples can be conserved for 24h in dark at 2-8℃, dry samples (before dissolved with n-hexane) is recommended.

(e) Remove part of the supernatant organic phase if the treated sample become emulsified, and keep in 60℃ water bath for 5-10min to eliminate the emulsion, then repeat the centrifuge step.

8.2 Tissue (chicken, chicken liver, pork, pig liver, fish and shrimp), honey, egg

-----Homogenize the tissue sample with homogenizer;

------Weigh 1.0±0.05g homogenized sample into a 50ml polystyrene centrifuge tube, then add 4ml of acetonitrile, vortex for 5min, then centrifuge for separation: *5min / 3000g / ambient temperature*;

-----Transfer 1ml of the supernatant organic phase into a 10ml clean glass test tube, dry with 50-60℃ water bath under nitrogen gas stream;

-----Add 1ml of n-hexane, vortex for 30s, then add 1ml of extraction solution(**solution 2**), vortex for 1min, then transfer the solution into a 2ml polystyrene centrifuge tube, then centrifuge for separation: 5min / 3000g / ambient temperature.

-----Remove the upper layer of organic phase, take 50μl of the lower layer phase for assay;

Notice: ***Increase the volume of n-hexane to 2-3ml when detecting samples high in fat.***

Dilution factor: 4

8.3 Milk (raw milk, UHT pure milk)

---Take 1ml of milk sample into a 2ml polystyrene centrifuge tube, then add 1ml of 1% trichloroacetic acid solution(**Solution 1**), vortex for 2min, then centrifuge for separation: *5min / 3000g / ambient temperature*;

------Transfer 200μl of the supernatant(*avoid the floating substance*) into a 2ml polystyrene centrifuge tube, add 800μl of extraction solution(**Solution 2**), mix completely.

-----Take 50μl of the lower layer phase for assay;

Dilute factor: 10

8.3 Feed sample

------Weigh 1.0±0.05g of the feed sample into a 50ml polystyrene centrifuge tube, add 10ml of wash solution(**Solution 3**) , vortex for 2min, then centrifuge for separation: 5min / 3000g / ambient temperature;

------Transfer 50μl of the supernatant(*avoid the floating substance*) into a 2ml polystyrene centrifuge tube, add 950μl of extraction solution(**Solution 2**), mix completely.

-----Take 50μl per well for assay.

Dilution factor: 200

9. Assay process

9.1 Notice before assay

9.1.1 Make sure all reagents and microwells are all at room temperature (20-25℃).

9.1.2 Return all the rest reagents to 2-8℃ immediately after used.

9.1.3 Washing the microwells correctly is an important step in the process of assay; it is the vital factor to the reproducibility of the ELISA analysis.

9.1.4 Avoid the light and cover the microwells during incubation.

9.2 Assay Steps

9.2.1 Take all reagents out at room temperature (20-25℃) for more than 30min, shake gently before use.

9.2.2 Get the microwells needed out and return the rest into the zip-lock bag at 2-8℃ immediately.

9.2.3 All reagents should be rewarmed before use.

9.2.4 **Number:** Number every microwell position and all

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standards and samples should be run in duplicate. Record the standards and samples positions.

9.2. 5 **Dilute the concentrated enzyme conjugate** : Dilute the concentrated enzyme conjugate(**Kit provided**) with the enzyme conjugate diluent(**Kit provided**) in the volume ratio 1:10(*e.g. 0.5ml of concentrated enzyme conjugate + 5ml of enzyme conjugate diluent*), mix thoroughly, ready for use.

Note:▲▲▲ *The diluted enzyme conjugate can not be preserved, use immediately.*

9.2.6 **Add standard solution/sample and diluted enzyme conjugate**: Add 50µl of standard solution(**Kit provided**) or prepared sample to corresponding wells, add 50µl of *diluted enzyme conjugate solution*(*see 9.2.5*) to each well, and then mix gently by shaking the plate manually and incubate for 30min at 25℃ with cover.

9.2.6 **Wash**: Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250µl diluted wash solution (**solution 3**) at interval of 10s for 4-5 times. Absorb the residual water with absorbent paper.

9.2.8 **Coloration**: Add 50µl of solution A(**Kit provided**) and 50µl of solution B(**Kit provided**) to each well. Mix gently by shaking the plate manually and incubate for 15 min at 25℃ with cover(*see 12.8*).

9.2.9 Measure: Add 50µl the stop solution(**Kit provided**) to each well. Mix gently by shaking the plate manually and measure the absorbance at 450nm against an air blank *(It's suggested measure with the dual-wavelength of 450/630nm. Read the result within 5min after addition of stop solution. We can also measure by sight without stop solution in short of the ELISA reader*)

10. Results

10.1 Percentage absorbance

The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

$$\text{Absorbance}\,(\%) = \frac{B}{B_0} \times 100\%$$

B ——absorbance standard (or sample)

B0 ——absorbance zero standard

10.2 Standard Curve

To draw a standard curve: take the absorbance value of standards as y-axis, semi logarithmic of the concentration of the florfenicol and thiamphenicol standards solution (ppb) as x-axis.

--- The florfenicol and thiamphenicol concentration of each sample(ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

Please notice:

For data reduction of the ELISA kits, special software has been developed, which can be provided onrequest.

11. Sensitivity, accuracy and precision

Test Sensitivity: **0.5ppb**

Tissue, honey, egg……....**0.2ppb**

Milk……..……**0.5ppb**

Feed ……**10ppb**

Accuracy

Tissue, honey, egg……..…100±30%

Milk……..……..……100±30%

Feed……..…….. 100±30%

Precision

Variation coefficient of the ELISA kit is less than 10%.

12. Notice

12.1 The mean values of the absorbance values obtained for the standards and the samples will be reduced if the reagents and samples have not been regulated to room temperature (20-25 ℃).

12.2 Do not allow microwells to dry between steps to avoid unsuccessful repetitiveness and operate the next step immediately after tap the microwells holder.

12.3 Shake each reagent gently before use.

12.4. Keep your skin away from the stop solution for it is 2M H_2SO_4.

12.5 Don't use the kits out of date. Don't exchange the reagents of different batches, or else it will drop the sensitivity.

12.6 Keep the ELISA kits at 2-8 ℃,do not freeze. Seal rest microwell plates Avoid straight sunlight for the standard sample and the colorless chromogenic reagent are sensitive to light.

12.7 Substrate solution should be abandoned if it turns colors. The reagents may turn bad if the absorbance value

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(450/630nm) of the zero standard is less than 0.5(A450nm<0.5).

12.8 The coloration reaction needs 10-15min after adding Solution A and Solution B. And you can prolong the incubation time ranges to 20min or more if the color is too light to be determined, never exceed 30min, On the contrary, shorten the incubation time properly.

12.9 The optimal reaction temperature is 25℃. Higher or lower temperature will lead to the changes of sensitivity and absorbance values

13. Storage condition and storage period

Storage condition: 2-8℃.

Storage period: 12 months.

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