Version 20-11/24

User's Manual

Tilmicosin ELISA Kit

REF **DEIA038**

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.

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PRODUCT INFORMATION

Intended Use

This kit can be used in quantitative and qualitative analysis of tilmicosin residue in animal tissue, liver, honey, egg, milk.

General Description

Tilmicosin is mainly used to against the bacterial and mycoplasma infection in livestock and poultry. It is also used to the additive of the feed of pig to promote the weight of the pig and the feed conversion rate.

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. It only needs 45min.

Principles of Testing

This kit is based on indirect-competitive ELISA technology. The microtiter wells are coated with coupling antigen. Tilmicosin 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 tilmicosin residue in it, after comparing with the Standard Curve, multiplied by the dilution factor, tilmicosin residue quantity in the sample can be calculated.

Reagents And Materials Provided

1. Microtiter plate with 96 wells coated with antigen
2. Standard solutions (6×1ml/bottle): 0ppb, 0.5ppb,1.5ppb, 4.5ppb,13.5ppb,40.5ppb
3. Spiking standard solution: (1ml/bottle) 1ppm
4. Enzyme conjugate 7ml, red cap
5. Antibody solution 7ml, green cap
6. Substrate solution A 7ml, white cap
7. Substrate solution B 7ml, red cap
8. Stop solution 7ml, yellow cap
9. 20×concentrated wash solution 40ml, transparent cap
10. 4×Concentrated extraction solution 50ml, blue cap

Materials Required But Not Supplied

1. Equipments

Microtiter plate spectrophotometer (450nm/630nm)

Rotary evaporator or nitrogen drying instruments

Homogenizer

Vortex mixer

Centrifuge

Analytical balance (inductance: 0.01g)

Graduated pipette: 10ml

Rubber pipette bulb

Volumetric flask: 100ml, 500ml

Glass tube: 10ml

Polystyrene Centrifuge tubes: 50ml

Micropipettes: 20μl-200μl, 100μl-1000μl, 250μl-multipipette

2. Reagents

Anhydrous sodium carbonate (AR)

Sodium bicarbonate (AR)

Methanol (AR)

Ethyl acetate (AR)

N-hexane (AR)

Deionized water

Storage

Storage condition: 2-8°C.

Storage period: 12 months.

Specimen Collection And Preparation

1. Notice and precautions before operation:

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

Make sure that all experimental tools are clean.

Keep untreated samples in freeze.

2. High detection limit: tissue (chicken, pork, beef, mutton, fish and shrimp)

Homogenize the sample with homogenizer;

Weigh 2.0±0.05g homogenized sample into a 50ml polystyrene centrifuge tube, then add 2ml of 0.1M (PH=10.6) CB buffer solution (solution 1), and 8ml of Ethyl acetate, vortex for 3min, then centrifuge for separation: 5min / 4000r/min / ambient temperature;

Transfer 4ml of the supernatant organic phase into a 10ml clean glass test tube, dry with 50-60°C water bath under nitrogen gas stream;

Add 1ml of n-hexane, vortex for 30s, then add 1ml of extraction solution(solution 4), vortex for 30s, then centrifuge for separation: 5min / 4000r/min / ambient temperature.

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

Dilution factor: 1

3. Low detection limit: tissue (chicken, duck, chicken liver, duck liver)

Homogenize the sample with homogenizer;

Weigh 1.0±0.05g homogenized sample into a 50ml polystyrene centrifuge tube, then add 4ml of wash solution (solution 5), vortex for 3min, then centrifuge for separation: 5min / 4000r/min / ambient temperature;

Transfer 200µl of supernatant, add 600µl of extraction solution (solution 4), vortex for 30s;

Take 50µl for assay;

Dilution factor: 20

4. Liver (chicken liver, pork liver)

Homogenize the sample with homogenizer;

Weigh 1.0±0.05g homogenized sample into a 50ml polystyrene centrifuge tube, then add 2ml of 0.5M (PH=10.6) CB buffer solution (solution 2), and 8ml of Ethyl acetate, vortex for 3min, then centrifuge for separation: 5min / 4000r/min / ambient temperature;

Transfer 2ml of the supernatant organic phase into a 10ml clean glass test tube, dry with 50-60°C water bath under nitrogen gas stream;

Add 1ml of n-hexane, vortex for 30s, then add 1ml of extraction solution(solution 4), vortex for 30s, then centrifuge for separation: 5min / 4000r/min / ambient temperature.

Remove the upper layer of organic phase, take 100µl of the lower layer phase and add 400µl extraction solution (solution 4), vortex for 30s.

Take 50µl for assay.

Dilution factor: 20

5. Honey

Weigh 2.0±0.05g honey sample into a 50ml polystyrene centrifuge tube, then add 2ml of 0.1M (PH=10.6) CB buffer solution (solution 1), and 8ml of Ethyl acetate, vortex for 3min, then centrifuge for separation: 5min / 4000r./min / ambient temperature;

Transfer 2ml of the supernatant organic phase into a 10ml clean glass test tube, dry with 50-60°C water bath under nitrogen gas stream;

Add 1ml of extraction solution(solution 4), vortex for 30s.

Take 50µl for assay;

Dilution factor: 2

6. Egg

Homogenize the egg sample with low speed.

Take 100µl of egg sample after homogenized, add 900µl egg diluents (solution 3), vortex for 30s.

Take 50µl for assay.

Dilution factor: 10

7. Milk

Take 100µl raw milk sample

Add 900µl milk diluents (solution 3), vortex for 30s.

Take 50µl for assay.

Dilute factor: 10

Reagent Preparation

Solution 1: 0.1M (PH=10.6) CB buffer solution

Dissolve 0.932g Anhydrous sodium carbonate and 0.1g Sodium bicarbonate with 100ml deionized water.

Solution 2: 0.5M (PH=10.6) CB buffer solution

Dissolve 4.66g Anhydrous sodium carbonate and 0.5g Sodium bicarbonate with 100ml deionized water.

Solution 3: Milk and egg diluents

Mix the extraction solution (solution 4) and methanol in the volume of 19:1.

Solution 4: Extraction solution

Dilute the 4× concentrated extraction solution with deionized water in the volume ratio of 1:3, which will be used for sample extraction. The diluted extraction solution can be conserved for 1 month at 4°C.

Solution 5: Wash solution

Dilute the 20× concentrated wash solution with deionized water in the volume ratio of 1:19, which will be used to rinse the plate. The diluted wash solution can be conserved for 1 month at 4°C.

Assay Procedure

1. Notice before assay

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

Return all the rest reagents to 2-8°C immediately after used.

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.

Avoid the light and cover the microwells during incubation.

2. Assay Steps

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

b. Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.

c. All reagents should be rewarmed before use.

d. Number: Number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.

e. Add standard solution/sample and enzyme conjugate and antibody solution: Add 50µl of standard solution(Kit provided) or prepared sample to corresponding wells, add 50µl of enzyme conjugate and 50µl

antibody solution, and then mix gently by shaking the plate manually and incubate for 30min at 25°C with cover.

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

g. 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°C with cover(see Precautions).

h. 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)

Calculation

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

2. Standard Curve

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

The Tilmicosin 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.

Performance Characteristics

Accuracy

High detection limit tissue: 90±20%

Low detection limit tissue: 90±20%

Liver (chicken liver, pork liver): 75±15%

Honey: 95±25%

Egg, Milk: 95±25%

Precision

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

Detection Limit

High detection limit tissue: 0.5ppb

Low detection limit tissue: 10ppb

Liver (chicken liver, pork liver): 10ppb

Honey: 1ppb

Egg, Milk: 5ppb

Sensitivity

0.5ppb

Specificity

Tilmicosin: 100%

Tylosin: <0.1%

Precautions

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°C).
2. Do not allow microwells to dry between steps to avoid unsuccessful repetitiveness and operate the next step immediately after tap the microwells holder.
3. Shake each reagent gently before use.
4. Keep your skin away from the stop solution for it is 2M H_2SO_4.
5. Don' t use the kits out of date. Don't exchange the reagents of different batches, or else it will drop the sensitivity.
6. Keep the ELISA kits at 2-8°C,do not freeze. Seal rest microwell plates Avoid straight sunlight for the standard sample and the colorless chromogenic reagent are sensitive to light.
7. Substrate solution should be abandoned if it turns colors. The reagents may turn bad if the absorbance value (450/630nm) of the zero standard is less than 0.5 (A450nm < 0.5).
8. The coloration reaction needs 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 25min, On the contrary, shorten the incubation time properly.
9. The optimal reaction temperature is 25°C. Higher or lower temperature will lead to the changes of sensitivity and absorbance values.