Version 12-06/24

User's Manual

Tiamulin ELISA Kit

REF **DEIA036**

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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 is intended for analysis of tiamulin residue in animal tissue, egg and feed

General Description

Tiamulin (previously thiamutilin) is a pleuromutilin antibiotic drug that is used in veterinary medicine particularly for pigs and poultry.

Tiamulin is a diterpene antimicrobial with a pleuromutilin chemical structure similar to that of valnemulin.

Principles of Testing

This kit uses an indirect competitive ELISA method. The conjugated antigen is pre-coated on the microwell strip. The residual tiamulin in the sample will compete with the conjugated antigen pre-coated on the microwell strip for anti-tiamulin antibodies. After adding the enzyme label, TMB substrate is used to develop the color. The absorbance value of the sample is negatively correlated with the content of the residue tiamulin contained in it. The content of the corresponding residue tiamulin can be obtained by comparing it with the standard curve.

Reagents And Materials Provided

1. Microplate: 8 wells per strip, 12 strips per plate
2. standard solution × 5 bottles, (1ml/bottle)
   0ppb, 0.03ppb, 0.09ppb, 0.27ppb, 0.81ppb
3. High concentration standard, 1ml, 100ppb
4. Enzyme conjugate, 7ml, red cap
5. Antibody working solution, 7ml, blue cap
6. Substrate A solution, 7ml, white cap
7. Substrate B solution, 7ml, black cap
8. Stop solution, 7ml, yellow cap
9. 20× Wash buffer, 40ml, white cap
10. 2× Reconstituted solution, 50ml, transparent cap

Materials Required But Not Supplied

Microplate reader, homogenizer, oscillator, centrifuge, graduated pipette, balance (sensitive to 0.01g), incubator;

Micropipette: single channel 20 ~ 200μl, single channel 100 ~ 1000μl, multi channel 30 ~ 300μl

Reagents: anhydrous methanol, anhydrous ethanol

Storage

1. Storage conditions: Store the kit at 2-8°C, do not freeze.
2. Shelf life: The shelf life of this product is 1 year.

Note: If there is a leak in the enzyme-linked immunosorbent assay (ELISA) plate vacuum packaging bag, the ELISA plate will still be effective and will not affect the experimental results.

Specimen Collection And Preparation

1. Note:

a. Disposable tips must be used in the experiment. The tips should be replaced when different reagents are aspirated.

b. Before conducting the experiment, it is necessary to check if all experimental apparatus is clean. If necessary, the apparatus should be cleaned to avoid contamination that may interfere with the experimental results.

2. Preparation of Sample dilution:

1× Sample dilution: Dilute the 2× reconstituted solution with deionized water at a 1:1 ratio.

3. Tissue: (chicken/pork/duck)

a. Weigh 1g ± 0.05g of homogenized tissue sample in a 10ml centrifuge tube. Add 2ml of anhydrous methanol. Shake for 3 minutes. Centrifuge at 4000r/min or higher for 5 minutes at room temperature;

b. Take 100µl of the supernatant. Add it to 900µl of Sample dilution, and shake well to mix;

c. Take 50µl for analysis.

Sample dilution factor: 30.

4. Feed:

a. Weigh 1g ± 0.05g ground feed sample in a 10ml or 50ml centrifuge tube. Add 5ml anhydrous methanol. Shake for 3 minutes, centrifuge at 4000r/min or above for 5 minutes at room temperature;

b. Take 100 µl supernatant, add it to 900µl sample diluent, shake well to mix;

c. Take 50 µl for analysis

Sample dilution factor: 50.

5. Egg:

1. Weigh 1 g ± 0.05 g of egg liquid sample in a 10ml centrifuge tube. Add 2ml anhydrous ethanol. Shake for 3 minutes, centrifuge at room temperature at 4000r/min for 5 minutes;
2. Take 100 µl of the supernatant, add it to 900µl of sample diluent, shake well to mix thoroughly;
4. Take 50 µl for analysis

Sample dilution factor: 30.

Assay Procedure

Note:

1. Before use, place the required reagents and strips at room temperature (20-25°C).
2. After use, immediately return all reagents to 2-8°C.
3. The reproducibility in ELISA analysis largely depends on the consistency of plate washing. Proper plate washing operation is crucial in the ELISA testing process.
4. Avoid exposure to light during all constant temperature incubation processes. Cover the microplate with a plate cover.

Procedure:

1. Bring all reagents and samples to room temperature for 30 minutes before use. Remember to shake each liquid reagent well before use.
2. Remove unused microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.Store them at 2-8°C.
3. Prepare the 1× Wash Buffer: Dilute 20× Wash Buffer with deionized water at a ratio of 1:19 (1 part concentrated wash buffer + 19 parts deionized water), or prepare the wash buffer according to the required amount.
4. Determine the number of wells for the assay run. Each sample and standard should have 2 parallel wells. Record the positions of the standard wells and sample wells.
5. Add Standard/Sample to each microwell (50 µl/well). Add Enzyme conjugate (50 µl/well). Add Antibody working solution (50 µl/well). Seal the plate with plate cover. React in 25°C environment for 30 minutes.
6. Remove the liquid from the wells and add 250µl of 1x wash buffer to each well. Wash the plate 4-5 times, with 15-30 second intervals, and pat dry with absorbent paper (any remaining bubbles after drying can be pierced with a clean pipette tip).
7. Add Substrate A solution (50µl/well), then add Substrate B solution (50µl/well), and gently mix. Allow the plate to develop color in darkness at 25°C for 15 minutes.
8. Measurement: Add Stop Solution (50µl/well), gently mix, and set the plate reader at 450nm (it is recommended to use a dual wavelength of 450/630nm for detection, with readings taken within 5 minutes). Measure the OD value of each well.

Calculation

There are two ways to determine the results. The first method can be used for rough judgment. The second method is used for quantitative determination. Note that the absorbance value of a sample is inversely related to the amount of tiamulin it contains.

1. Method 1:

By comparing the average absorbance value of the sample with the standard value, the concentration range (ng/ml) can be obtained. Assume that the absorbance value of sample 1 is 0.3 and that of sample 2 is 0.7. The absorbance values of the standard solution are: 0ppb——2.043; 0.03ppb——1.716; 0.09ppb——1.015; 0.27ppb——0.44; 0.81ppb—— —0.064. Then the concentration range of sample 1 is 0.27ppb ~ 0.81ppb; the concentration range of sample 2 is 0.09ppb ~ 0.27ppb. Multiplied by the corresponding dilution factor is the actual concentration of tiamulin in the sample.

2. Method 2 (quantitative analysis):

a. Calculation of absorbance: The absorbance of a standard or sample is equal to the average of the absorbance values of the standard or sample (dual holes) divided by the absorbance value of the first standard (0 standard), and then multiplied by 100%

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

B—average absorbance value of standard solution or sample solution

B_0—average absorbance value of 0ng/ml standard solution

b. Standard curve:

Draw the standard curve using the absorbance (%) of the standard as the y-axis and the semi-logarithm of the concentration of the tiamulin standard (ng/ml) as the x-axis. Plug the absorbance of the sample into the standard curve. Read the concentration corresponding to the sample from the standard curve. Multiplied by its corresponding dilution factor is the actual concentration of tiamulin in the sample.

If the professional analysis software of the kit is used for calculation, it will be more convenient for accurate and rapid analysis of a large number of samples.

Detection Limit

Tissue, eggs: 1ppb

Feed: 2 ppb

Sensitivity

0.03ppb

Specificity

Tiamulin: 100%

Recovery

Tissue, feed, eggs: 90±30%

Precautions

1. If the room temperature is lower than 25°C or the reagents and specimens have not returned to room temperature (20-25°C), the OD values of all standards will be low.
2. If the plate wells become dry during the plate washing process, the standard curve will not be linear and the repeatability will be poor. Therefore, you should proceed to the next step immediately after washing the plate and patting it dry.
3. Mixing must be uniform, otherwise the repeatability of the experiment will be affected.

4. The reaction stop solution is 2M sulfuric acid, avoid contact with skin.
5. Do not use test kits that have expired. Dilution or mixed use will cause changes in sensitivity and OD value. Do not exchange reagents from different lot numbers in the box.
6. Put unused microplates into ziplock bags and reseal them; standard substances and colorless chromogens are sensitive to light, so avoid direct exposure to light.
7. If any color of the chromogenic solution indicates deterioration, it should be discarded. When the absorbance value of standard 1 is less than 0.5, it means that the reagent may have deteriorated.
8. The optimal reaction temperature of this kit is 25°C. Temperature that is too high or too low will cause changes in the detection absorbance value and sensitivity.