# Competitive Enzyme Immunoassay Kit for Quantitative Analysis of Tylosin

## 1. Background

Tylosin is a macrolide antibiotic, which is mainly applied as antibacterial and anti-mycoplasma. Strict MRLs haven been established since this drug may lead to serious side effect in certain groups.

This kit is a new product based on ELISA technology, which is fast, easy, accurate and sensitive compared with common instrumental analysis and only needs 1.5 hours in one operation, it can considerably minimize operation error and work intensity.

## 2. Test Principle

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

## 3. Applications

This kit can be used in quantitative and qualitative analysis of tylosin residue in animal tissue (chicken, pork, duck) and milk, honey, egg, etc.

## 4. Cross-reactions

Tylosin……..100%

Tilmicosin……<2%

## 5. Materials Required

### 5.1 Equipments:

----Microtiter plate spectrophotometer (450nm/630nm)

----Rotary evaporator or nitrogen drying instruments

----homogenizer

----Shaker

----Centrifuge

----Analytical balance (inductance: 0.01g)

----Graduated pipette: 10ml

----Rubber pipette bulb

----Volumetric flask: 10ml

----Polystyrene centrifuge tubes: 50ml

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

250μl-multipipette

### 5.2 Reagents:

**----** Sodium hydroxide (NaOH, AR)

----Sodium bicarbonate ($NaHCO_3$, AR)

---- Sodium carbonate ($NaCO_3$, AR)

----Trichloroacetic acid (AR)

---- Acetonitrile (AR)

----Ethyl acetate (AR)

-----n-Hexane (AR)

----Deionized water

## 6. Kit Components

- Microtiter plate with 96 wells coated with antigen
- Standard solutions(5 bottles,1ml/bottle)
  **0ppb, 0.5ppb, 1.5ppb, 4.5ppb, 13.5ppb**
- Spiking standard control: (1ml/bottle) **1ppm**
- Enzyme conjugate 1ml……..…red cap
- Antibody solution 7ml……green cap
- Solution A 7ml……white cap
- Solution B 7ml...……..red cap
- Stop solution 7ml……yellow cap
- 20×concentrated wash solution 40ml
  ……..…transparent cap
- 4×concentrated extraction solution 50ml
  ……blue cap

## 7. Reagents Preparation:

**Solution 1:** 0.1mol/L NaOH solution

Weigh 0.4g NaOH to 100ml deionized water and mix completely.

**Solution 2**: 1mol/L NaOH solution

Weigh 4g NaOH to 100ml deionized water and mix up completely.

**Solution 3**: Carbonate buffer salt

**Solution** 1: 0.2M PB

Dissolve 51.6g of $Na_2HPO_4 \cdot 12H_2O$, 8.7g of $NaH_2PO_4 \cdot 2H_2O$ with deionized water and dilute to 1000ml.

**Solution** 2: Extraction solution

Dilute the 2×concentrated extraction solution with

deionized water in the volume ratio of 1:1(*e.g. 10ml of 2×extraction solution + 10ml of deionized water*), which will be used for sample extraction, *this solution can be stored at 4℃ for 1 month*.

**Solution** 3: Wash solution

Dilute the 20×concentrated wash solution with deionized water in the volume ratio of 1:19(*e.g. 5ml of 20×wash solution + 95ml of deionized water*), which will be used for washing the plates. *This solution can be stored at 4℃ for 1 month*.

## 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 instruments are clean.*

*(c) Keep tissue sample in freeze.*

*(d) Prepared sample should be used for assay at once.*

### 8.2 Animal tissue (chicken, pork, etc)

----Homogenize the sample with homogenizer;

----Take 2.0±0.05g of homogenate into a 50ml polystyrene centrifuge tube; add 2ml of 0.2M PB (**solution** 1) , shake to dissolve, and then add 8ml of ethyl acetate and shake fiercely for 3min;

----Centrifuge for separation: 3000g / ambient temperature / 5min.

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

----Dissolve the dry leftover with 1ml of n-hexane, vortex for 30s to dissolve, and then add 1ml of extraction solution (**solution** 2), vortex for 1min. centrifuge for separation: 3000g / ambient temperature / 5min

----Remove the supernatant n-hexane phase; take 50µl of the substrate aqueous phase for assay.

***Dilution factor: 1***

### 8.2 Milk

----Take 100µl of raw milk sample, mix with 900µl of extraction solution (**solution** 2), and mix completely.

----Take 50µl of the prepared solution for assay.

***Dilution factor: 10***

## 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 The diluted wash solution should be rewarmed to be at room temperature before use.

9.2.4 **Number:** Numbered every microwell positions and all standards and samples should be run in duplicate. Record the standards and samples positions.

9.2.5 **Add standard solution/sample and antibody solution**: Add 50µl of standard solution((**kit provided**)) or prepared sample to corresponding wells. Add 50µl of antibody solution(**kit provided**). Mix gently by shaking the plate manually and incubate for 30min at 37℃ with cover.

9.2.6 **Wash**: Remove the cover gently and pure the liquid out of the wells and rinse the microwells with 250µl diluted wash solution (**solution** 3) at interval of 10s for 4-5times. Absorb the residual water with absorbent paper (the rest air bubble can be eliminated with unused tip).

9.2.7 **Add enzyme conjugate**: Add 100µl of enzyme conjugate solution(**kit provided**) to each well, mix gently and incubate for 30min at 37℃ with cover. *Repeat the wash step again*.

9.2.8 **Coloration**: Add 50µl of solution A(**kit provided**) and 50µl of solution B(**kit provided**) to each well. Mix gently and incubate for 15min at 37℃ with cover.

9.2.9 **Measure**: Add 50µl of the stop solution(**kit provided**) to each well. Mix gently and measure the absorbance at 450nm (It's suggested measure with the dual-wavelength of 450/630nm. Read the result within

5min after adding stop solution).

**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.

$$\text{Absorbance (\%)} = \frac{B}{B0} \times 100\%$$

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 tylosin standards solution (ppb) as x-axis.

----The tylosin 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:**

special software has been developed for data analysis, which can be provided on request.

**11. Sensitivity, accuracy and precision**

Test Sensitivity: **1.5ppb**

**Detection limit:**

Animal tissue……1.5ppb

Milk……..15ppb

**Accuracy:**

Animal tissue……80±15%

Milk……80±10%

**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 reproducibility 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 0.5M $H_2SO_4$ solution.

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 during all incubations. Covering the microtiter plates is recommended.

12.7 Substrate solution should be abandoned if it turns colors. The reagents may be turn bad if the absorbance value (450/630nm) of the zero standard is less than 0.5 (A450nm<0.5).

12.8 The coloration reaction need 15min after the addition of 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 37℃. Higher or lower temperature will lead to the changes of sensitivity and absorbance values.

**13. Storage**

Storage condition: 2-8℃.

Storage period: 12 months**.**