Tylosin ELISA Test Kit

1. Principle

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

2. Technical specifications

Sensitivity : 1.5 ppb

Detection limit:

Meat,liver.....3 ppb

Honey.....1.5 ppb

Because of some interference in honey and milk, the detection limit is 4 ppb.

Recovery rate:

Meat ..... 90%±15%

liver..... 80%±10%

Honey..... 85%±15%

Cross-reaction rate:

Tylosin 100%

3. Components

1） Micro-well strips: 12 strips with 8 removable wells each

2） 6× standard solution (1 mL each): 0ppb,1.5ppb,4.5ppb,13.5ppb,40.5ppb,121.5ppb

3） Enzyme conjugate (7 mL) .....red cap

4） Antibody working solution (7 mL) ..... blue cap

5） Substrate A solution (7 mL) .....white cap

6） Substrate B solution (7 mL).....black cap

7） Stop solution (7 mL) .....yellow cap

8） 20× concentrated washing buffer (40 mL) .....white cap

9） 5× concentrated redissolving solution (50 mL) .....transparent cap

4. Materials required but not provided

1) **Equipments:** microplate reader, printer, homogeniser, nitrogen-drying device, vortex, centrifuge, measuring pipets, balance (a reciprocal sensibility of 0.01 g)

2) **Micropipettors:** single-channel 20-200 µL and 100 to 1000 µL, and multi-channel 250 µL

3) **Reagents:** NaOH, CHCl3, Acetonitrile(CH_3CN), Methanol,HCl, deionized water

Skype: missyan819 **Tel:** 86-755-21568988 **Fax:** 86-755-28938800
Email:info@lsybt.com,lvshiyuan711@gmail.com **Website**: http://www.lsybt.com
Address: Rm.507, No.2, longgang Overseas Venture Park,Shenzhen,China. 518172

5. 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 different reagents.

2） Before the experiment, each experimental equipment 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:

1 0.1 M NaOH : dissolve 0.4 g NaOH in deionized water to 100 mL.

2. Sample extract solution:(CH_3CN-0.1M HCl-Methanol mixture): 84ml CH_3CN +16ml 0.1M HCl +18ml Methanol

3. 2×concentrated redissolving solution is mixed with deionized water at 1:1 (1 mL 2× concentrated redissolving solution+ 1 mL deionized water), ued for sample redissolving.

5.1 Meat,liver

1 Homogenize the sample.

2 Take 2± 0.05 g of the homogenized sample into 50 mL centrifugal tube, add 8 mL Sample extract solution, shake it with Oscillator for 5 min, centrifuge at above 4000 r/min at room temperature(25 ℃) for 10 min.

3 Take 2 mL of the supernatant,add 1ml 0.1M NaOH, mix evenly,add 3ml CHCl3, shake it with Oscillator for 5 min;

4 centrifuge at above 4000 r/min at room temperature(25 ℃) for 10 min,remove the upper phase,take the lower phase into a new vessel, blow to dry by nitrogen or air at 56 ℃.

5. Dissolve the dry residues in 1 mL of the diluted redissolving solution,mix for 30s,

6 Take 50 µL for further analysis.

Fold of dilution of the sample: 2 ***detection limit:3ppb***

5.2 Honey

1 Put 1.0 ± 0.05 g honey into 50ml centrifuge tube, add 2 mL deionized water,rock it with votex for 2min,then add 10ml CHCL3,shake up and down for 5min,

2 centrifuge at above 4000 r/min at room temperature (25 ℃) for 10 min. remove the upper phase,take the lower phase into a new vessel, blow to dry by nitrogen or air at 56 ℃.

3. Dissolve the dry residues in 1 mL of the diluted redissolving solution,mix for 30s,

3 Take 50 µL for further analysis.

Fold of dilution of the sample: 1 ***detection limit:1.5ppb***

6. ELISA procedures

6.1 Instructions

1 Bring all reagents and micro-well strips to the room temperature (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 procedures of ELISA.

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.

6.2 Operation procedures

1 Take out the kit from 4 ℃ environment. Take out all the necessary reagents from the kit and place at the room temperature (20-25 ℃) for at least 30 min. Note that each reagent must be shaken to mix evenly before use.

2 Take the required micro-well strips and plate frames. Re-sealed the unused microplate, stored at 2-8 ℃, not frozen.

3 Solution preparation: dilute 40 mL of the concentrated washing buffer (20×concentrated) with the distilled or deionized water to 800 mL (or just to the required volume) for use.

4 Numbering: number the micro-wells according to samples and standard preparation; each 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 into every well ,then add 50 µL of antibody working solution into each well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, and incubate at 37 ℃ for 40 min.

6 Pour the liquid out of the wells ,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 10 s,after the last time,flap to dry on absorbent paper (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 B solution into each well. Mix gently by shaking the plate manually, and incubate at 37 ℃ for 20 min at dark for coloration.

9 Determination: add 50 µL stop solution into each well. Vortex evenly. 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).

7. 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 Tylosin in the sample.

7.1 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 0.5, and that of the sample Ⅱ is 1, while those of the standard solutions are as the followings: 1.675 for 0 ppb, 1.398 for 1.5 ppb, 1.197 for 4.5 ppb, 0.8720 for 13.5 ppb, 0.510 for 40.5 ppb and 0.213 for 121.5 ppb, accordingly the concentration range of the sample Ⅰ is 40.5 to 121.5 ppb, and that of the sample Ⅱ is 4.5 to 13.5 ppb.

7.2 Quantitative determination

The mean values of the absorbance values is obtained for the average OD value (B) of the sample and the standard solution divided by the OD value (B0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is,

$$\text{Percentage of absorbance value} = \frac{B}{B0} \times 100\%$$

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

B0—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 semilogarithmic values of the Tylosin 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 actual concentration of Tylosin 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)

8. Precautions

1） The room temperature below 20 ℃ 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;

3） Mix every reagent and reaction mixture evenly and wash the microplate thoroughly, otherwise there will be the undesirable reproducibility;

4） The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin;

5） 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;

6） 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;

7） Discard the colouration solution with any color that indicates the degeneration of this solution. The detecting value of standard solution 1（0 ppb） of less than 0.5 indicates its degeneration;

8） Colouration time is 15 min after the addition of the substrate A and then the B solution, if the color is light, prolong the time, don't exceed 30min;

9） 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.

9. Storage and expiry date

Storage: store at 2-8 ℃, not frozen.

Expiry date: 12 months; please check date of production before use.

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Green Earth depends on everyone' efforts
"Build of green Earth needs the cooperation of you and me"

Skype: missyan819 **Tel:** 86-755-21568988 **Fax:** 86-755-28938800
Email:info@lsybt.com,lvshiyuan711@gmail.com **Website**: http://www.lsybt.com
Address: Rm.507, No.2, longgang Overseas Venture Park,Shenzhen,China. 518172