Tylosin ELISA Test Kit

1. Principle

This test kit is based on the competitive enzyme immunoassay for the detection of Tylosin in the meat and liver (chicken, pork), honey. The coupling antigen is pre-coated on the micro-well stripes. The Tylosin in the sample and the coupling antigen pre-coated on the micro-well stripes compete for the anti - Tylosin antibody. 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 the Tylosin in it. This value is compared to the standard curve and the Tylosin concentration is subsequently obtained.

2. Technical specifications

Sensitivity: 1.5 ppb

Detection limit

Meat .....3 ppb
Liver .....3 ppb
Honey.....1.5 ppb

Recovery rate

Meat ..... 80%±15%
Liver ..... 75%±10%
Honey..... 80%±15%

3. Components

1） Micro-well strips: 12 strips with 8 removable wells each
2） 6× standard solution (1 ml each): 0 ppb, 1.5 ppb, 4.5 ppb, 13.5p pb ,40.5 ppb,121.5 ppb
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） 2× concentrated redissolving solution (50 ml)..... transparent cap

4. Materials required but not provided

1) **Equipments:** microplate reader (450 nm / 630 nm), homogenizer, oscillator, votex, centrifuge, and balance (a sensibility reciprocal of 0.01 g)
2) **Micropipettors:** single-channel 20 to 200 µl and 100 to 1000 µl, and multi-channel 250 µl;
3) **Reagents:** ethanenitrile (CH_3CN), NaCl, deionized water, 0.1 M HCl, NaOH, $CHCl_3$

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 absorbing different reagents;
2) Before the experiment, each experimental utensil must be checked to 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-8% NaCl solution: add 0.4 g NaOH and 100 ml 8% NaCl, mix evenly.
2) Acetonitrile-HCl solution : $V_{acetonitrile}$: V_{H2O} = 84 : 16 (add 84 ml acetonitrile and 16 ml 1 M HCl, mix evenly)
3) The 2× concentrated redissolving solution is mixed with the deionized water at 1:2(1 ml concentrated redissolving solution + 1 ml deionized water)

5.1 Liver and meat sample

1. Homogenize the sample.
2. Take 2± 0.05 g of the homogenized sample into tube, add 8 ml of acetonitrile-HCl solution, shake properly with oscillator for 5 min
3. Centrifuge at above 3000 r/min at room temperature (20 - 25 ℃) for 10 min.
4. Take 2 ml supernatant, add 2 ml of 0.1 M NaOH - 8% NaCl solution, mix properly, add 8 ml CH_3CN to extract, shake with oscillator for 5 min
5. Centrifuge at above 3000 r/min at room temperature (20-25 ℃) for 10 min, remove the upper layer, take the lower, blow to dry with nitrogen
6. Dissolve the dry residues in 1 ml of the diluted redissolving solution
7. Take 50 µl for analysis.

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

5.2 Honey

1. Weigh 1± 0.05 g honey into 50 ml centrifuge tube, add 2 ml deionized water, vortex for 2 min to dissolve, then add 10 ml CH_3CN, shake upside down for 5 min
2. Centrifuge at above 3000 r/min at room temperature (20 – 25 ℃) for 10 min, remove the upper layer, take organic phase (the lower), blow to dry with nitrogen
3. Dissolve the dry residue in 1 ml of the diluted redissolving solution
4. Take 50 µl for analysis.

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

6. ELISA procedures

6.1 Instructions

1. Bring all reagents and micro-well strips to the room temperature (20 – 25 ℃) before use.
2. Return all reagents to 2 to 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 the refrigerated environment. Take out all the necessary reagents from the kit and place at the room temperature (20 to 25 ℃) for at least 30 min. Note that each liquid 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 to 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 solution; each sample and standard solution should be performed in duplicate; record their positions.
5. Add 50μl of the sample and 50 μl of the standard solution into each well, and add 50 μl enzyme conjugate and then 50 μl of the antibody 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. Take out the microplate, add 250 μl/well of washing buffer for 10 sec, repeat four to five times, then flap to dry (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;
8. Determination: add 50 μl of the stop solution into each well. Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450 nm to determine the OD value (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 content of Tylosin.

7.1 Qualitative determination

The concentration range (ng/ml) can be obtained from the comparison the average OD value of the sample with that of the standard solution. Assuming that the OD value of the sample Ⅰ is 0.50, and that of the sample Ⅱ is 1.0 , while those of the standard solutions are as the followings: 1.675 for 0ppb, 1.398 for 1.5 ppb, 1.197 for 4.5 ppb, 0.872 for 13.5ppb, 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. (multiplied by the corresponding dilution fold).

7.2 Quantitative determination

The mean values of the absorbance values is equivalent to the percentage of the average OD value (B) of the sample and the standard solution divided by the OD value (B_0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is,

$$\text{Percentage of absorbance value} = \frac{B}{B_0} \times 100\%$$

B—the average (double wells) OD value of the sample or the standard solution

B_0—the average OD value of the 0ng/ml standard solution

Draw the standard curve with the absorption percentages of the standard solutions and the semilogarithm 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 Tylosin concentration 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 to 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 the 0 standard solution of less than 0.5 indicates its degeneration
8. 20 min for coloration after addition of subtrate A and B, if the coloration is light, prolong time and don't exceed 30 min.
9. The optimum reaction temperature is 37 ℃, 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 to 8 ℃, not frozen.

Expiry date: 12 months; date of production is on the box

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"Build of green Earth needs the cooperation of you and me"