VERSION 1.01

TYLOSIN ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Tylosin ELISA Test Kit

Catalogue Number. IP100019

Principle

This test kit is based on the competitive enzyme immunoassay for the detection of Tylosin in the sample. The coupling antigens are pre-coated on the micro-well stripes. The Tylosin in the sample and the coupling 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 the Tylosin in it. This value is compared to the standard curve and the Tylosin concentration is subsequently obtained.

Technical specifications

Sensitivity: 1ppb
Detection limit: Pork, Chicken (method 1) about 20ppb, Pork, Chicken (method 2) about 1ppb, Honey about 1ppb, Raw milk, reduced milk about 15ppb
Note: ppb= ng/ml or ng/g
Cross-reaction rate: Tylosin 100%
Recovery rate: 90±30%

Components

1	Micro-well strips	12 strips with 8 removable wells each	
2	5× standard solution (1mL each)	0ppb, 1ppb, 3ppb, 9ppb, 27ppb	
3	Enzyme conjugate	12ml	red cap
4	Antibody working solution	7ml	blue cap
5	Substrate A	7ml	white cap
6	Substrate B	7ml	black cap
7	Stop solution	7ml	yellow cap
8	20× concentrated washing buffer	30ml	white cap
9	5× concentrated redissolving solution	10ml	transparent cap

Materials required but not provided

Equipments: microplate reader (450nm, 630nm), printer, homogenizer, nitrogen-drying device, vortex, shaker, centrifuge (4000g and above), measuring pipets, balance (a reciprocal sensibility of 0.01 g), incubator (25℃), water bath, timer;
Micropipettors: single-channel 20-200 µL, 100-1000 µL, and eight-channel 30～300 µl;
Reagents (AR): 12MHCl, NaOH, ethyl acetate, n-Hexane, deionized water, $Na_2HPO_4.12H_2O$, $NaH_2PO4.2H_2O$.

Sample pre-treatment

Instructions

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 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) Redissolving solution: 1 part 5× concentrated redissolving solution + 4 parts deionized water.
2) 0.2M Phosphate buffer: weigh 51.6g $Na_2HPO_4.12H_2O$ and 8.7g $NaH_2PO4.2H_2O$, add 1L deionized water to dissolve evenly..
3) Washing buffer: 1 part 20× concentrated washing buffer + 19 parts deionized water.
4) 0.1 M NaOH: dissolve 0.8g NaOH in 200ml deionized water.
5) 1M HCl: Weigh 1ml 12MHCl, add 11ml deionized water to dissolve.

Samples preparation

a) Chicken, pork
Method 1

1) Weigh 1± 0.05 g homogenized tissue sample into 50ml plastic centrifuge tube;
2) Add 2ml deionized water, shake strongly for 2min (or use vortex for 1min);
3) Centrifuge at above 4000 g at room temperature for 10min;
4) Take 100ul supernatant, add 400ul Redissolving solution, mix evenly for 10s;
5) Take 50ul to test.
Fold of dilution of the sample: 15

Method 2

1) Weigh 1± 0.05 g homogenized tissue sample into 50ml plastic centrifuge tube, add 1ml 0.2M Phosphate buffer to dissolve completely, then add 8ml ethyl acetate, shake or vortex for 3min, mix it evenly;
2) Centrifuge at above 3000 g at room temperature for 5min;
3) Take 4ml supernatant into a 10ml clean glass centrifuge tube, blow to dry in 50-60℃ water bath by nitrogen-drying device;
4) Add 1ml n-Hexane, vortex for 30s, then add 0.5ml Redissolving solution, vortex for 1min, mix evenly, centrifuge at above 3000 g at room temperature for 5min;
5) Discard up-layer organic phase, take 50ul down-layer liquid to test.
Fold of dilution of the sample: 0.5

b) Honey

1) Weigh 2± 0.05 g honey sample into 50ml plastic centrifuge tube, add 2ml 0.1M NaOH to dissolve completely, then add 6ml ethyl acetate, shake for 5min, mix evenly;
2) Centrifuge at above 3000 g at room temperature for 5min;
3) Take 3ml up-layer organic phase into 10ml clean dry glass tube, blow to dry in 50-60℃ water bath by nitrogen-drying device;
4) Add 0.5ml Redissolving solution, vortex for 1min, be static at room temperature for 5min, vortex for 1min again, mix evenly;
5) Take 50ul down-layer liquid to test.

Fold of dilution of the sample: 0.5

c) Raw milk, reduced milk

Reduced milk: weigh 1g±0.05g milk powder, add 7ml deionized water, shake and dissolve, become reduced milk.
1) Take the collected raw milk/reduced milk, thaw and return to room temperature for above 30min;
2) Put tips into down-layer of raw milk/reduced milk, take out 1ml sample into 2ml centrifuge tube(Note: do not take up-layer cream);
3) Add 50ul 1M HCl, shake strongly for 1min (or vortex for 30s);
4) Centrifuge at above 4000 g at room temperature for 10min;
5) Take 100ul up-layer clear liquid into another centrifuge tube (do not take up-layer cream), then add 900ul Redissolving solution,shake strongly for 1min (or vortex for 30s);
6) Take 50ul liquid to test.
Fold of dilution of the sample: 10

ELISA procedures

Instructions

1) Bring all reagents and micro-well strips to the room temperature (20-25 ℃) before use;
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 ELISA the procedures;
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.

Operation procedures

1. Bring test kit to the room temperature (20-25 ℃) for at least 30 min, note that each reagent must be shaken to mix evenly before use, put the required micro-well strips into plate frames. Re-sealed the unused microplate, store at 2-8 ℃, not frozen.
2. Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate, record their positions.
3. Add 50 µL of the sample or standard solution into separate duplicate wells; then add 50 µL of the antibody working solution into each well, mix gently by shaking the plate manually. Seal the microplate with the cover membrane, and incubate at 25 ℃ for 30min in dark.
4. Pour liquid out of microwell, flap to dry on absorbent paper, add 250 µL/well of washing buffer to wash microplate for 15-30 s, then take out and flap to dry with absorbent paper, repeat 3-4 times. (If there are the bubbles after flapping, cut them with the clean tips).
5. Add 100ul enzyme conjugate, mix gently by shaking the plate manually. Seal the microplate with the cover membrane, and incubate at 25 ℃ for 30min in dark. Washing as step 4.
6. Coloration: add 100ul mixture of substrate A solution and substrate B solution into each well (Note: mix substrate A solution and substrate B solution at 1:1, use the mixture in 10min, do not use metal to contain or stir, to avoid substrate invalid). Mix gently by shaking the plate manually, seal the microplate with the cover membrane then incubate at 25 ℃ for 15 min at dark for coloration.
7. Determination: add 50 µL of the stop solution into each well. Mix gently by shaking the plate manually. Stop successfully when substrate color from blue to yellow. Recommend to read the OD value at the dual-wavelength 450/630 nm within 5 minutes.

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 concentration.

Qualitative determination

The concentration range (ng/mL) of Tylosin 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 0.3, and that of the sample Ⅱ is 1.0, the OD value of standard solutions is: 2.243 for 0ppb, 1.816 for 1ppb, 1.415 for 3ppb, 0.74 for 9ppb, 0.313 for 27ppb, accordingly the concentration range of the sample Ⅰ is 9 to 27ppb, and that of the sample Ⅱ is 1 to 3ppb.

Quantitative determination

The mean values of the absorbance values obtained for the percentage of 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,

Percentage of absorbance value = $(B / B_0) \times 100\%$

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

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

Draw the standard curve with the absorption percentages of the standard solution and the semilogarithm values of the Tylosin standard solution (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, finally obtaining the Tylosin concentration in the sample.

Precautions

1. The room temperature below 25 ℃ 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; So continue to next step immediately after washing.
3. Mix evenly, otherwise there will be the undesirable reproducibility.
4. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin.
5. 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 lots to use.
6. Put the unused microplate into an auto-sealing bag to re-seal it. The standard solution and the colourless color former is light sensitive, and thus they cannot be directly exposed to the light.
7. Discard the colouration solution with any color that indicates the degeneration of this solution. The detecting value of the standard solution 1(0 ppb) of less than 0.5 indicates its degeneration.

Storage: store at 2-8 ℃, not frozen.
Expiry date: 12 months; date of production is on box.