VERSION 1.01

TETRACYCLINES ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Tetracyclines ELISA Test Kit

Catalogue Number. IP100006

Principle

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

Technical specifications

Sensitivity: 0.05ppb
Incubation Temperature: 25℃
Incubation Time: 30min～15min
Detection limit: Tissue (Chicken): Tetracyclines 0.4ppb, Minocycline 0.4ppb, RoliTetracyclines 0.4ppb, Aureomycin 0.4ppb, Demethylchlor Tetracyclines 1.2ppb, Terramycin 0.8ppb, Doxycycline 1ppb; Pork, Honey: Tetracyclines 0.5ppb, Minocycline 0.5ppb, RoliTetracyclines 0.5ppb, Aureomycin 0.5ppb, Demethylchlor Tetracyclines 1.5ppb, Terramycin 1ppb, Doxycycline 1.2ppb.
Cross-reaction rate: Tetracyclines 100%, Minocycline 125%, RoliTetracyclines 110%, Aureomycin 100% Demethylchlor Tetracyclines 35%, Terramycin 58%, Doxycycline 45%
Recovery rate: Chicken, Pork, Honey 85±20%

Components

1	Micro-well strips	12 strips with 8 removable wells each	
2	Standard Concentrate (81ppb)	1ml	Black cap
3	High-concentration standard (100ppb)	1ml	black cap
4	Enzyme conjugate	7ml	white cap
5	Antibody working solution	7ml	white cap
6	Substrate A	7ml	white cap
7	Substrate B	7ml	black cap
8	Stop solution	7ml	yellow cap
9	20× concentrated washing buffer	15ml	white cap
10	10× sample extract	50ml * 2	transparent cap
11	Standard redissolving solution	15ml *2	blue cap

Materials required but not provided

Equipments: microplate reader, rotary evaporator/nitrogen-drying device, homogenizer, oscillator, centrifuge, balance (a sensibility reciprocal of 0.01 g), measuring pipets, incubator.

Micropipettors: single-channel 20-200µL, 100-1000µL, and multi-channel 30～300µl;
Reagents: methanol

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) Sample extract A
Dilute 50ml 10X sample extract with 450ml deionized water or dilute 10X sample extract with deionized water at 1:9 (If there is crystals in the 10X sample extract, first dissolve then dilute)
2) Sample extract B
Take 50ml methanol, mix with 450ml Sample extract A, or mix methanol and Sample extract A at 1:9
3) Sample extract C
Take 100ml methanol, mix with 270ml Sample extract A, or mix methanol and Sample extract A at 10:27

Samples preparation

a) Chicken

1) Take 1± 0.05 g of the homogenized sample into 50 mL centrifuge tube, add 8mL Sample extract A, shake for 3min, centrifuge at above 4000 r/min at room temperature (20 - 25 ℃) for 10 minutes.
2) Take 50ul supernatant for analysis.
Fold of dilution of the sample: 8

b) Pork

1) Take 1± 0.05 g of the homogenized sample into 50 mL centrifuge tube, add 10mL Sample extract B, shake for 3min, centrifuge at above 4000 r/min at room temperature (20 - 25 ℃) for 10 minutes.
2) Take 50ul supernatant for analysis.
Fold of dilution of the sample: 10

c) Honey

1) Take 1± 0.05 g of the homogenized sample into 50 mL centrifuge tube, add 10mL Sample extract C, shake for 3min, centrifuge at above 4000 r/min at room temperature (20 - 25 ℃) for 10 minutes.
2) Take 50ul supernatant for analysis.
Fold of dilution of the sample: 10

ELISA procedures

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.

Operation procedures

1. Take out all the necessary reagents from the kit and place at the room temperature (20 to 25 ℃) for at least 30 minutes. Note that each liquid reagent must be shaken to mix evenly before use.
2. Standard preparation

Standard 1: Standard redissolving solution	**0ppb**
Standard 2: Mix 600ul Standard redissolving solution and 300ul Standard 3	**0.05ppb**
Standard 3: Mix 600ul Standard redissolving solution and 300ul Standard 4	**0.15ppb**
Standard 4: Mix 600ul Standard redissolving solution and 300ul Standard 5	**0.45ppb**
Standard 5: Mix 600ul Standard redissolving solution and 300ul Standard 6	**1.35ppb**
Standard 6: Dilute 50ul Standard Concentrate (81ppb) with 950ul Standard redissolving solution	**4.05ppb**

3. Take the required micro-well strips and plate frames. Re-sealed the unused microplate, stored at 2-8 ℃, not frozen.
4. Washing buffer preparation: dilute 15 mL of the 20× concentrated washing buffer with the deionized water at 1:19 (1 part 20× concentrated washing buffer + 19 parts deionized water). Or prepare washing buffer as quantity needed.
5. Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate; record their positions.
6. Add 50μL of the sample or standard solution to separate duplicate wells, and add 50ul Enzyme conjugate 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 25℃ for 30 minutes.
7. Pour liquid out of microwell, add 250 μL/well of washing buffer for 15-30 seconds, repeat four to five times, then flap to dry (if there are the bubbles after flapping, cut them with the clean tips).
8. Coloration: add 50 μL of the substrate A and then 50 μL of the substrate B into each well. Mix gently by shaking the plate manually, and incubate at 25 ℃ for 15 minutes at dark for coloration.
9. 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 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 content of Tetracyclines in the sample.

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 0.3, and that of the sample Ⅱ is 1.0, while those of the standard solutions are as the followings: 2.243 for 0 ppb, 1.816 for 0.05 ppb, 1.415 for 0.15 ppb, 0.74 for 0.45 ppb, 0.313 for 1.35 ppb and 0.155 for 4.05 ppb, accordingly the concentration range of the sample Ⅰ is 1.35 to 4.05ppb, and that of the sample Ⅱ is 0.15 to 0.45 ppb.

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,

Percentage of absorbance value = (B / B_0) × 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 solutions and the semilogarithm values of the Tetracyclines 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 Tetracyclines 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)

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.
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. 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.

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