VERSION 1.01

DOXYCYCLINE ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Doxycycline ELISA Test Kit

Catalogue Number. IP100029

Principle

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

Technical specifications

Sensitivity: 0.2 ppb
Incubation Temperature: 25℃
Incubation Time: 30min—15min
Detection limit: Tissue about 30ppb, Honey about 50ppb, Milk about 100ppb
Note: ppb=ng/mL or ng/g
Cross-reaction rate:Doxycycline100%, Chlortetracycline400%, Oxytetracyline200%, Tetracycline300%
Recovery rate:
Tissue 90±15%

Components

1	Micro-well strips	12 strips with 8 removable wells each
2	10× standard solution (0.5mL each)	0 ppb, 20ppb, 60ppb, 180ppb, 540ppb
3	Enzyme conjugate	7ml
4	Antibody working solution	7ml
5	Substrate A	7ml
6	Substrate B	7ml
7	Stop solution	7ml
8	20× concentrated washing buffer	30ml
9	20× concentrated redissolving solution	10ml

Materials required but not provided

Equipments: microplate reader (450nm, 630nm), rotary evaporator/nitrogen-drying device, homogenizer, oscillator, centrifuge (4000g and above), balance (a sensibility reciprocal of 0.01 g), measuring pipets, incubator (adjustable 25℃、37℃、60℃),timer
Micropipettors: single-channel 20~200 µL and 100~1000 µL, and multi-channel 30~300 µL;
Reagents: deionized water.

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) Dilute 20× concentrated redissolving solution with deionized water at 1:19(1 part concentrated re-dissolving solution + 19 parts deionized water).
2) Washing buffer: 1 part 20× concentrated washing buffer + 19 parts deionized water

Samples preparation

a) Tissues

1. Take 1± 0.01 g of the homogenized sample into 10 mL centrifuge tube, add 5 mL deionized water, shake with oscillator for 2min, centrifuge at above 4000 r/min at room temperature (20 - 25 ℃) for 10 minutes.
2. Take 500ul supernatant, add 500ul diluted redissolving solution, shake with oscillator for 10min;
3. Take 50 µL for analysis.

Fold of dilution of the sample: 12

b) Honey

1) Take 1± 0.01g honey sample into 10 mL centrifuge tube; Add 2ml deionized water, shake with oscillator fully for 1 min to dissolve; take 100ul dissolved solution, add 400ul diluted redissolving solution, oscillator for 10S to mix it evenly;
2) Take 50ul for analysis immediately.

c) Milk

1) Take 50ul liquid sample into 1950ul diluted redissolving solution; oscillator fully for 1min evenly;
2) Take 50ul for analysis immediately.

Fold of dilution of the sample: 40

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. Dilute the 5 concentrated standard solution separately: take 5 pieces of 2ml centrifuge tube, mark 0、2、6、18、54ppb accordingly, add 900μL the diluted redissolving solution into each tube, then add the five 10X concentrated standard solution into above 5 tubes accordingly, 100ul/tube. The 5 diluted standard solutions will be: 0—0、2—20、6—60、18—180、54—540.
3. Take the required micro-well strips and plate frames. Re-sealed the unused microplate, stored at 2-8 ℃,not frozen.
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 or standard solution to separate duplicate wells, then add enzyme conjugate, 50 μL each well, then add 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 ℃ at dark for 30 minutes.
6. Pour liquid out of microwell, add 250 μL/well of washing buffer for 15-30 seconds, repeat three to four times, then flap to dry (if there are the bubbles after flapping, cut them with the clean tips).
7. Coloration: add 100 μL mixture of the substrate A and substrate B into each well (Note: mix Substrate A and Substrate B at 1:1, the mixture should be used in 10min, never use metal container or metal to stir the solution, otherwise the substrate may be invalid.). Mix gently by shaking the plate manually, and incubate at 25 ℃ for 15 minutes at dark for coloration.
8. Determination: add 50 μL of the stop solution into each well (The substrate color from blue to yellow, it means the stop succeeds). 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 Doxycycline 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 0ppb, 1.816 for 20ppb, 1.415 for 60ppb, 0.74 for 180ppb, 0.313 for 540ppb, accordingly the concentration range of the sample Ⅰ is 180 to 540ppb, and that of the sample Ⅱ is 20 to 60 ppb.

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 solutions and the semilogarithm values of the Doxycycline 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 Doxycycline concentration in the sample.
Using the professional analyzing software of this kit will be more convenient for the accurate and rapid an alysis 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.