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Doxycycline ELISA Kit

rev 04/21

(Catalog # E4613-100, 100 assays, Store at 4°C)

I. Introduction:

Doxycycline is a synthetic derivative of oxytetracycline. It acts as a broad spectrum antibiotic and matrix metallo-proteinases (MMP) inhibitor. It is also useful in studies involving wound healing and tissue remodeling. Common side effects include diarrhea, nausea, vomiting, a red rash, and an increased risk of a sunburn. Use during pregnancy or in young children may result in permanent problems with the teeth including changes in their color. Standard techniques/instruments (HPLC or GC-MS) are utilized to detect Doxycycline. However, these techniques are complex, expensive, laborious, and time-consuming. Immunoassay techniques, such as ELISAs are commonly preferred as a simple, reliable and rapid method for the quantification of Doxycycline in various samples. BioVision's Doxycycline ELISA Kit is a competitive-based ELISA that can be used for the determination of this antibiotic in tissue, honey, and serum.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Doxycycline
Detection Range: 0.1 – 8.1ppb
Sensitivity: 0.1ppb
Cross reaction: Doxycycline 100%, tetracycline 150%, minocycline 92%, pyrithione 76%, chlortetracycline 75%, Demethylchromycin 70%, oxytetracyline 83%

III. Sample Type:

Tissue, Honey, Serum

IV. Kit Contents:

Components	E4613-100	Part No.
Micro ELISA Plate	8 X 12 Strips	E4613-100-1
Standard (S0 – S6)	1 ml X 7	E4613-100-2
Enzyme Conjugate	7 ml	E4613-100-3
Antibody working solution	7 ml	E4613-100-4
Substrate A	7 ml	E4613-100-5
Substrate B	7 ml	E4613-100-6
Stop Solution	7 ml	E4613-100-7
Wash Buffer (20X)	15 ml	E4613-100-8
Sample Extract A (20X)	15 ml	E4613-100-9
Sample Extract B (2X)	50 ml X 2	E4613-100-10
Sample Diluent (20X)	10 ml	E4613-100-11
Plate Sealer	3	E4613-100-12

V. User Supplied Reagents and Equipment:

- Reagents: deionized water, NaOH, Acetonitrile
- Microplate reader capable of measuring absorbance at 450 nm
- Clean eppendorf tubes and graduated cylinders for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment. Opened kit may be stable for 1 month at 4°C.

VII. Reagent and Sample Preparation:

Note: Bring all reagents to room temperature (20-25°C) 30 minutes before use.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Wash Buffer (1X):** Dilute 15 mL of Wash buffer (20X) with deionized water at 1:19 (1 part Wash buffer (20X) + 19 parts deionized water). Alternatively, prepare washing buffer as quantity needed.

2. **Ready to use Standards Concentration:**

Standards	S0	S1	S2	S3	S4	S5	S6 (High Standard)
concentration (ppb)	0	0.1	0.3	0.9	2.7	8.1	1 ppm

3. **Sample Preparation:**
Note: The prepared sample maybe stored for up to one day at 2-8°C.

Sample pre-treatment: The following method must be used for pre-treatment of any kind of sample:
Note: Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents.

Solution preparation before sample pre-treatment:

1) **Sample extract A (1X):** 1 part of sample extract A (20X) + 19 parts of deionized water
2) **Sample extract B (1X):** 1 part of sample extract B (2X) + 1 part of deionized water
3) **1M NaOH solution:** Weigh 4 g NaOH, add deionized water to 100ml
4) **Sample diluent (1X):** 1 part of 20x sample diluent + 19 parts of deionized water

Sample Preparation and pre-treatment (for Tissue and Honey):

Detection limit: 4ppb

- Take 2 ± 0.05 g of the homogenized tissue/honey sample into 50 mL centrifuge tube, add 3 mL **Sample extract A (1X)**, mix for 3 min.
- Add 600 µl **1M NaOH solution** and 2.4 ml **Sample extract B (1X)**, mix for 3 min, and centrifuge at above 4000 r/min at room temperature (20 - 25 ℃) for 5 minutes.
- Take 50 µl of upper layer clear solution and add 450 µl **Sample diluent (1X)**, mix evenly.
- Take 50 µl above mixed solution for analysis.
- **Fold of dilution of the sample: 40**

Sample preparation for Serum

Detection limit: 2ppb

- Take 1 ml serum sample in 50 ml centrifuge tube, add 1 ml **Acetonitrile**, and mix for 3 mins. Centrifuge at above 4000 r/min at room temperature (20 - 25 ℃) for 5 minutes
- Take 100 µl of upper layer clear solution, add 400 µl **Sample diluent (1X)**. Mix them evenly.
- Take 50 µl above mixed solution for analysis.
- **Fold of dilution of the sample: 10**

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Add 50 µL of the **sample or standards** to separate duplicate wells, and add 50 µL **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.
2. Aspirate liquid out of microwells, add 250 µL/well of **wash buffer (1X)** for 15-30 seconds. Repeat four to five times, tap to dry (if there are the bubbles after tapping, remove them with the clean tips).
3. 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.
4. 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 reading the OD value at the wavelength 450 nm within 5 minutes).

IX. Calculation:

Quantitative determination

The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

Absorbance Value (%) = B/B_0 X 100%

B: The average absorbance value of the sample or standard

B_0: The average absorbance value of the 0 ppb standard

To draw a standard curve: Take the absorbency value of standards as y-axis, logarithmic of the concentration of the Doxycycline standards solution (ppb) as x-axis. The Doxycycline concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

Qualitative determination:

The concentration range (ng/mL) can be obtained by comparing the average OD value of the sample with that of the standard solution.

Example: 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 0.1ppb, 1.415 for 0.3ppb, 0.74 for 0.9ppb, 0.313 for 2.7ppb and 0.155 for 8.1ppb, accordingly the concentration range of the sample Ⅰ is 2.7 to 8.1ppb, and that of the sample Ⅱ is 0.3 to 0.9ppb.

X. Related Products:

- Doxycycline hyclate (Cat. No. 2209)
- Anti-Doxycycline Antibody (24E2) (Cat. No. A1302)
- Streptomycin ELISA Kit (Cat. No. E4272-100)
- Gentamicin (serum/urine) ELISA Kit (Cat. No. K4315-100)
- Kanamycin ELISA Kit (Cat. No. K4210-100)
- Quinolone ELISA Kit (Cat. No. E4530-100)