Doxycycline ELISA Kit

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

I. Introduction:
Doxycycline is a synthetic derivative of oxytetracycline. Acts as a broad spectrum antibiotic and matrix metallo-proteinases (MMP) inhibitor. 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, milk and serum.

II. Application:
This ELISA kit is used for in vitro quantitative determination of Doxycycline
Detection Range: 0.2 – 5.4 ppb
Sensitivity: 0.2 ppb
Detection limitation: 2 ppb for serum, 5 ppb tissue, honey and milk, 10 ppb for milk
Cross reaction: Doxycycline 100%, tetracycline, minocycline 100%, chlortetracycline 180%, oxytetracycline 40%

III. Sample Type:
Tissue, honey, milk, serum

IV. Kit Contents:

<table>
<thead>
<tr>
<th>Components</th>
<th>E4613-100</th>
<th>Part No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro ELISA Plate</td>
<td>8 X 12 Strips</td>
<td>E4613-100-1</td>
</tr>
<tr>
<td>Standard (10X) (S0 – S4)</td>
<td>0.5 ml X 5</td>
<td>E4613-100-2</td>
</tr>
<tr>
<td>Enzyme Conjugate (11X)</td>
<td>0.7 ml</td>
<td>E4613-100-3</td>
</tr>
<tr>
<td>Enzyme conjugate dilution</td>
<td>7 ml</td>
<td>E4613-100-4</td>
</tr>
<tr>
<td>Substrate A</td>
<td>7 ml</td>
<td>E4613-100-5</td>
</tr>
<tr>
<td>Substrate B</td>
<td>7 ml</td>
<td>E4613-100-6</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>7 ml</td>
<td>E4613-100-7</td>
</tr>
<tr>
<td>Wash Buffer (20X)</td>
<td>30 ml</td>
<td>E4613-100-8</td>
</tr>
<tr>
<td>Redissolving Solution (20X)</td>
<td>10 ml</td>
<td>E4613-100-9</td>
</tr>
</tbody>
</table>

V. User Supplied Reagents and Equipment:
- Chemicals: deionized water, HCl, N,N-Dimethylformamide (DMF)
- 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. Redissolving Solution: mixed Redissolving Solution (20X) with deionized water at 1:19 (1 ml concentrated redissolving solution + 19 ml deionized water)
2. Wash Buffer: If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 30 ml of Wash Buffer (20X) into 570 ml deionized or distilled water to prepare 600 ml of Wash Buffer (1X). Keep it at 4°C for one month.
3. Enzyme Conjugate: take 1 part 11X Concentrated Enzyme conjugate, add 10 parts Enzyme conjugate dilution, dilute at 1:10.
4. Standards Concentration: Take 5 centrifuge tube (2 ml) and mark 0 · 0.2 · 0.6 · 1.8 · 5.4 ppb accordingly. Add 450 ul of the diluted redissolving solution into each tube, then add 50 ul of 10X concentrated standard solution into above 5 tubes accordingly.

<table>
<thead>
<tr>
<th>Standards</th>
<th>S0</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X Standards (ppb)</td>
<td>0</td>
<td>2</td>
<td>6</td>
<td>18</td>
<td>54</td>
</tr>
<tr>
<td>Working concentration (ppb)</td>
<td>0</td>
<td>0.2</td>
<td>0.6</td>
<td>1.8</td>
<td>5.4</td>
</tr>
</tbody>
</table>

V. Sample Preparation:
Note: The prepared sample may be stored for up to one day at 2-8°C.

FOR RESEARCH USE ONLY! Not to be used on humans.

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A. Tissue (Method 1)

Take 1 g of the homogenized tissue sample into 10 mL centrifuge tube, add 5 mL deionized water, shake with oscillator for 2 min, centrifuge at above 4000 g for 5 minutes. Take 500 µl up-layer clear liquid, add 500 µl diluted redissolving solution, shake with oscillator for 2 min. Take 50 µl up-layer liquid for analysis. (Dilution factor: 12)

B. Tissue (Method 2)

Take 1 g of the homogenized tissue sample into 10 mL centrifuge tube, add 1 ml N,N-Dimethylformamide (DMF), shake with oscillator for 5 min, to make sample completely dispersed, fully contact with the organic phase. Centrifuge at above 4000 g for 10 minutes. Take 100 µl up-layer clear liquid, add 900 µl diluted redissolving solution, shake with oscillator for 10 min. Take 50 µl for further analysis. (Dilution factor: 20)

C. Honey

Take 1 g honey sample into 10 mL centrifuge tube; Add 2 ml deionized water, shake with oscillator fully for 1 min to dissolve; take 100 µl dissolved solution, add 400 µl diluted redissolving solution, oscillator for 10 sec to mix it evenly. Take 50 µl for further analysis. (Dilution factor: 10)

D. Milk (Method 1)

Thaw the collected liquid milk sample, then put at room temperature for 30 min. Put tips in the down-layer of milk, take 1 ml sample into 2 ml centrifuge tube (note: do not take the up-layer cream). Add 50 µl 1M HCl, shake strongly for 1 min (or oscillator for 30 s). Centrifuge at above 4000 r/min at room temperature (20-25°C) for 10 minutes. Take up-layer clear liquid 50 µl into another clean centrifuge tube (note: do not take the up-layer cream), add 450 µl diluted redissolving solution, shake strongly for 1 min (or oscillator for 30 s). Take 50 µl for analysis immediately. (Dilution factor: 10)

E. Milk (Method 2)

Take 50 µl liquid sample into 1950 µl diluted redissolving solution; oscillator fully for 1 min evenly. Take 50 µl for analysis immediately. (Dilution factor: 40)

F. Serum

Take 100 µl sample, add 700 µl diluted redissolving solution, shake with oscillator for 10 s. Take 50 µl for analysis immediately. (Dilution factor: 8)

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.

1. Prepare all reagents, samples and standards as instructed in section VII.
2. Add 50 µl of the sample and standard solution to separate duplicate wells; Then 50 µl enzyme conjugate into each well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, and incubate at 25°C for 30 min.
3. Wash the microplate with the washing buffer at 250 µl/well for 4-5 times. Each time soak the well with the washing buffer for 15-30 sec, flap to dry with absorbent paper (if there are the bubbles after flapping, cut them with the clean tips).
4. 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 10 min, 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°C for 15 minutes at dark for coloration.
5. Add 50 µl of the stop solution into each well. Mix gently by shaking the plate manually. Read the OD value at the dual-wavelength 450/630 nm within 5 min.

IX. Calculation:

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.

\[
\text{Absorbance Value (\%) } = \frac{B}{B_0} \times 100\%
\]

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 Fluoroquinolones standards solution (ppb) as x-axis. The Fluoroquinolones 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.
Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

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)