Doxycycline (DOX) ELISA Kit

Catalog No: abx365114

Size: 96T

Detection Limit: Serum, Liver, Urine - 20 ng/ml; Egg, Raw Milk, Muscle - 10 ng/ml

Sensitivity: 0.5 ng/ml (ppb)

Cross-reactivity: Chlorotetracycline – 100%; Tetracyclines – 100%; Oxytetracycline – 60%

Storage: Store at 2-8°C.

Application: The quantitative detection of Doxycycline (DOX) in Serum, Liver, Urine, Egg, Raw Milk, and Muscle samples.

Principle of the Assay: This kit is based on competitive enzyme-linked immuno-sorbent assay technology. An antigen is pre-coated onto a 96-well plate. Standards, test samples, and biotin-conjugated reagent are added to the wells and incubated. A competitive inhibition reaction takes place between the pre-coated DOX and the DOX in the sample with the biotin-labelled antibody. The HRP-conjugated reagent is then added, and the whole plate is incubated. Unbound conjugates are removed using wash buffer at each stage. TMB substrate is used to quantify the HRP enzymatic reaction. After TMB substrate is added, only wells that contain sufficient DOX will produce a blue colored product, which then changes to yellow after adding the acidic stop solution. The intensity of the color yellow is inversely proportional to the DOX amount in the samples. The OD is measured spectrophotometrically at 450 nm in a microplate reader, from which the concentration of DOX can be calculated.

Kit Components

- Pre-coated 96-Well Microplate: 12 x 8
- Wash Buffer (20X): 25 ml
- Standard (20X): 6 × 1 ml (0 ng/ml, 10 ng/ml, 30 ng/ml, 90 ng/ml, 270 ng/ml, 810 ng/ml)
- Standard Diluent: 60 ml
- Sample Diluent Buffer (20X): 25 ml
- Detection Reagent A: 7 ml
- Detection Reagent B: 12 ml
- TMB Substrate A: 6 ml
- TMB Substrate B: 6 ml
- Stop Solution: 6 ml
- Plate Sealer: 3
- Hermetic Bag: 1

Materials Required But Not Provided

- Incubator
- Multi and single channel pipettes and sterile pipette tips
- Squirt bottle or automated microplate washer
- 1.5 ml tubes
- Distilled water
- Absorbent filter papers
- 100 ml and 1 liter graduated cylinders
- Microplate reader (wavelength: 450 nm)
- ELISA Shaker

Reagents Required But Not Provided

- Trichloroacetic acid
- Acetonitrile

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Protocol

A. Reagent Preparation

1 M Trichloroacetic Acid Solution: Dilute 16.4 g of Trichloroacetic acid with 100 ml of deionized water.

Standard Working solutions: Dilute each standard vial 20-fold (1/20) with Standard Diluent (i.e. add 1 ml of concentrated standard into 19 ml Standard diluent). **Do not vortex.** Mix with gentle agitation prior to use. Standard Working solutions should be used within 12 hours of preparation.

Sample Diluent Working Solution: Dilute Sample Diluent 20-fold (1/20) with deionized water (i.e. add 25 ml of Sample Diluent Buffer (20X) into 475 ml deionized water). Mix thoroughly.

Wash Buffer: Dilute the concentrated Wash Buffer 20-fold (1/20) with distilled water (i.e. add 25 ml of Wash Buffer (20X) into 475 ml of distilled water). If crystals have formed in the Wash Buffer (20X), warm to room temperature and mix gently until the crystals have completely dissolved.

B. Sample Preparation

Sample Pretreatment

1. **1 M Trichloroacetic Acid Treatment**
    a. **Muscle, raw milk, egg, urine and serum samples:** Remove fat from samples if applicable, homogenize solid samples manually or mechanically, then add 50 mg of sample to a centrifuge tube. Add 0.1 ml of 1 M Trichloroacetic Acid Solution and mix fully.
    b. **Liver samples:** Remove fat from samples if applicable, homogenize solid samples manually or mechanically, then add 50 mg of sample to a centrifuge tube. Add 0.2 ml of 1 M Trichloroacetic Acid Solution and mix fully.
2. **Acetonitrile Treatment**
    a. **Serum, Liver, Urine, Egg, Raw Milk, and Muscle samples:** Add 1 ml of Acetonitrile and vortex to mix fully. Centrifuge at 4000 rpm for 5 minutes at room temperature. Take 100 µl of the supernatant to a new centrifuge tube, and add 900 µl of Sample Diluent Working Solution. Vortex for 1 minute. Take 50 µl for immediate assay.

Sample Dilution Factor: 20

Sample:	Detection Limit:
Muscle, Raw milk and Egg	10 ng/ml (ppb)
Urine, Liver and Serum	20 ng/ml (ppb)

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Notes

- Store frozen samples undiluted. Once ready to analyze, thaw samples and dilute .
- Fresh samples, or recently obtained samples, are recommended to prevent protein degradation and denaturation that may lead to erroneous results.
- NaN_3 cannot be used as a test sample preservative, since it inhibits HRP.
- If possible, prepare solid samples using sonication and/or homogenization, as lysis buffers may interfere with the kit's performance.
- If a sample is not indicated in the manuals applications, a preliminary experiment to determine the suitability of the kit will be required.

C. Assay Protocol

Equilibrate the kit components and samples to room temperature prior to use. It is recommended to measure in duplicate.

1. Set sample and standard wells on the pre-coated microplate and record their positions.

   Add the solution to the bottom of each well without touching the side walls. Pipette the standards and samples up and down to mix before adding to the wells. Avoid foaming or bubbles.
2. Aliquot 50 µl of prepared sample into the sample wells.
3. Aliquot 50 µl of each diluted standard into the respective standard wells.
4. Aliquot 50 µl of Detection Reagent A into all wells. Gently tap the plate to mix, or use a microplate shaker.
5. Cover the plate with a plate sealer and incubate for 30 mins in the dark at 25°C.
6. Remove the cover and discard the liquid. Wash the plate 4 times with 1X Wash Buffer. *Fill each well completely with Wash buffer (260 µl) using a multi-channel Pipette or autowasher (0.5 mins soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.*
7. Aliquot 100 µl of Detection Reagent B to each well then gently tap the plate to mix, or use a microplate shaker. Cover the plate with a plate sealer and incubate for 30 mins in the dark at 25°C.
8. Remove the cover, discard the liquid, and repeat the wash process as described above, 4 times.
9. Aliquot 50 µl of TMB Substrate A and 50 µl of TMB Substrate B into each well. Cover the plate with the plate sealer and gently tap the plate to mix thoroughly. Incubate in the dark at 25°C for 10-15 mins. The incubation time is for reference only, the optimal time should be determined by end user.
10. Aliquot 50 µl of Stop Solution into each well. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
11. Ensure that there are no fingerprints or water on the bottom of the plate, and that there are no bubbles in the wells. Measure the OD at 450 nm immediately.

Data Analysis:

$$\text{Absorbance (\%)} = \frac{\text{Average Sample or Standard Absorbance}}{\text{Average Blank (0 ng/ml Standard) Absorbance}} \times 100$$

The standard curve can be plotted as the relative Absorbance (%) of each reference standard solution (X), against the

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respective concentration of each standard solution (Y). The concentration of the samples can be interpolated from the standard curve. *If the samples measured were diluted, multiply the concentrations from interpolation by the dilution factor* to obtain the concentration before dilution.

Precautions:

- Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
- Do not leave the wells uncovered for extended periods between incubations. The addition of reagents for each step should not exceed 10 mins.
- Ensure that the plate is properly sealed or covered during the incubation steps, and that the time and temperature are controlled. Do not reuse pipette tips and tubes.
- Do not use expired components, or components from a different kit.
- The TMB substrate should be used under sterile conditions, and light exposure should be minimized. Unused substrate should be colorless, or a very light yellow in appearance. Do not discard any residual solution back into the vial.
- Please note that this kit is optimized for detection of native samples, rather than recombinant proteins or synthetic chemicals. We are unable to guarantee detection of recombinant proteins, as they may have different sequences or tertiary structures to the native protein.

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.

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