Tetracycline (TCs) ELISA Kit

Catalog No.: abx364845

Size: 96T

Storage: Store the kit at 2-8°C.

Application: For quantitative detection of TCs in Tissue, Liver, Honey, Milk, Milk Powder, Egg, and Urine.

Sensitivity: 0.3 ng/ml (ppb)

Detection Limit: Tissue, Liver, Egg, Honey, Milk – 12 ng/ml, Milk Powder – 24 ng/ml, Urine – 3 ng/ml

Cross-reactivity: Tetracyclines, Chlortetracycline – 100%, Oxytetracycline – 51%, Doxycycline – 8.5%.

Introduction: Tetracycline is an antibiotic used to treat a number of infections. It is first-line therapy for Rocky Mountain spotted fever (Rickettsia), Lyme disease (B. burgdorferi), Q fever (Coxiella), psittacosis, and Mycoplasma pneumoniae and to eradicate nasal carriage of meningococci. Tetracyclines have a broad spectrum of antibiotic action. Bacteria usually acquire resistance to tetracycline from horizontal transfer of a gene that either encodes an efflux pump or a ribosomal protection protein. Efflux pumps actively eject tetracycline from the cell, preventing the buildup of an inhibitory concentration of tetracycline in the cytoplasm.

Principle of the Assay

This kit is based on competitive enzyme-linked immuno-sorbent assay technology. TCs is pre-coated onto a 96-well plate. The standards and samples and a biotin conjugated antibody specific to TCs are added to the wells and incubated. After washing away the unbound conjugates, avidin conjugated to Horseradish Peroxidase is added to each microplate well. After TMB substrate solution is added only wells that contain TCs will produce a blue colour product that changes into yellow after adding the stop solution. The intensity of the yellow colour is inversely proportional to the TCs amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of TCs can be calculated.

Kit components

1. One pre-coated 96 well plate
2. 1000 ng/ml (1.0 ppm) Standard: 1 ml
3. Empty Standard vials: (0 ng/ml, 0.3 ng/ml, 0.9 ng/ml, 2.7 ng/ml, 8.1 ng/ml, 24.3 ng/ml)
4. Detection Reagent A: 5.5 ml
5. Detection Reagent B: 11 ml
6. TMB Substrate A: 6 ml
7. TMB Substrate B: 6 ml
8. Stop solution: 6 ml
9. Wash buffer (20X): 40 ml
10. Reconstitution Buffer (5X): 50 ml
11. Plate sealer: 3
12. Hermetic bag: 1

Materials Required But Not Provided

1. 37°C incubator
2. Microplate reader (450 nm)
3. High-precision pipette and sterile pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5 ml EP tubes to prepare samples
7. Absorbent filter papers
8. 100 ml and 1 L graduated cylinders

Reagents Required But Not Provided

1. Hydrochloric acid
2. Methanol anhydrous
3. Deionized water

Protocol

A. Preparation of sample and reagents

1. Reagent Preparation

- **Extraction Solution**
  Dilute 4.3 ml of HCl with 45.7 ml of deionized water to a final volume of 50 ml. Add 450 ml of Methanol anhydrous and mix well.

- **Reconstitution Buffer (1X)**
  Dilute the 5× Reconstitution Buffer 5-fold with deionized water (i.e. dilute 10 ml of 5X Reconstitution buffer in 40 ml deionized water) to make the 1X Reconstitution Buffer solution. The 1X solution can be stored at 4°C for up to one month.

- **Wash Buffer (1X)**
  Dilute the 20X Wash Buffer 20-fold with deionized water to prepare the 1X Wash Buffer solution (e.g., dilute 40 ml of 20X Reconstitution Buffer in 760 ml deionized water to prepare 800 ml of 1X Wash Buffer).

2. Sample Preparation

Store samples to be assayed within 24 hours at 2-8°C. Alternatively, aliquot and store at -20°C or -80°C for long term. Avoid repeated freeze-thaw cycles.

- **Tissue, Liver and Egg:** Remove fat from the sample (except for egg samples). Homogenize with a tissue homogenizer, mixing fully. Weigh 1 ± 0.05 g of homogenate and add 2 ml of Extraction Solution and mix for 2 min with a vortex. Centrifuge at 4000 rpm for 5 min at room temperature. Aliquot 50 µl of supernatant into a clean tube. Add 950 µl of 1X Reconstitution solution and mix fully. Take 50 µl for detection.
  Note: Dilution factor: 40, Detection limit: 12 ng/ml.

- **Honey:** Weigh 1 ± 0.05 g of honey, add 1 ml of Extraction Solution and mix for 2 min with a vortex. Aliquot 50 µl of the above liquid into a clean tube. Add 950 µl of 1X Reconstitution Buffer and mix fully. Take 50 µl for detection.
  Note: Dilution factor: 40, Detection limit: 12 ng/ml.

- **Urine:** If urine is not clear, filter the urine and centrifuge at 4000 rpm for 10 minutes and collect the supernatant. Repeat this process until the urine becomes clear. Take 20 µl of the supernatant, add 180 µl of 1X Reconstitution Buffer and mix fully. Take 50 µl for detection.
  Note: Dilution factor: 10, Detection limit: 3 ppb

- **Milk:** Take 1 ml of fresh milk sample and add 2ml of Extraction Solution and mix for 2 min with a vortex. Centrifuge at 4000 rpm for 5 min at room temperature. Aliquot 50 µl of supernatant into a clean tube. Add 950 µl of 1X Reconstitution Buffer and mix fully. Take 50 µl for detection.
  Note: Dilution factor: 40, Detection limit: 12 ppb.

- **Milk Powder:** Weigh 1 ± 0.05 g of milk powder and add to 4 ml of Extraction Solution, then vortex for 2 minutes. Centrifuge at 4000 rpm for 5 min at room temperature. Aliquot 50 µl of supernatant into a clean tube. Add 950 µl of 1X Reconstitution solution and mix fully. Take 50 µl for detection.
  Note: Dilution factor: 80, Detection limit: 24 ppb

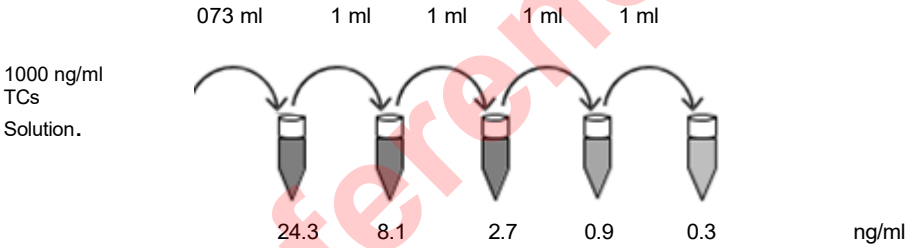
Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results. It is recommended to store samples to be used within 5 days at 4°C, within 1 month at -20°C and within 2 months at -80°C.
- Samples should be clear and transparent. Samples must be diluted so that the expected concentration falls within the kit's range.
- Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used. Samples that contain NaN_3 cannot be detected as it interferes with HRP.
- Always use non-pyrogenic, endotoxin-free tubes for blood collection.

3. Preparation of working standard solutions

Bring all reagents to room temperature for at least 30 minutes. The low concentration standard solutions should be prepared just before carrying out the assay, as they are unstable.

- Add 3 ml of Reconstitution Solution to the 0 ng/ml standard vial to prepare the working 0 ng/ml standard solution.
- Add 2 ml of Reconstitution Solution to each of the 0.3 ng/ml, 0.9 ng/ml, 2.7 ng/ml and 8.1 ng/ml standard vials. Add 2.93 ml of Reconstitution Solution to the 24.3 ng/ml standard vial.
- Take 73 µl of 1000 ng/ml standard and add to the 24.3 ng/ml standard vial, then mix thoroughly to prepare the working 24.3 ng/ml standard solution. Add 1 ml of working 24.3 ng/ml standard solution to the 8.1 ng/ml standard vial, and mix thoroughly to prepare the working 8.1 ng/ml standard solution.
- Add 1 ml of the working 8.1 ng/ml standard solution to the 2.7 ng/ml vial, and so on.



B. Assay Procedure

Bring all reagents to room temperature prior to use.

1. Set standard, sample and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate.
2. Add 50 µl of the standard solutions into the standard wells.
3. Add 50 µl of prepared sample into sample wells
4. Immediately add 50 µl of Detection Reagent A to all wells. Add the solution at the bottom of each well without touching the side wall.
5. Cover the plate with the plate sealer. Tap the plate gently to ensure thorough mixing. Incubate at 37°C for 30 minutes in the dark.
6. Remove the cover, and wash the plate 5 times. *Discard the solution without touching the side walls. Blot the plate on an absorbent material. Add 300 µl of wash buffer to each well and soak for at least 30 sec. Discard the contents and clap the plate on absorbent filter papers or other absorbent material.*
7. Add 100 µl of Detection Reagent B to all wells. Add the solution at the bottom of each well without touching the side wall.
8. Cover the plate with the plate sealer. Tap the plate gently to ensure thorough mixing. Incubate at 37°C for 30 minutes in the dark.
9. Remove the cover, and wash the plate 5 times as described in step 6.
10. Add 50 µl of Substrate Reagent A and 50 µl of Substrate Reagent B to all wells. Mix thoroughly and incubate at 37°C in the dark for 10-20 minutes.
11. Add 50 µl of Stop Solution to all wells and mix thoroughly.
12. Read and record the absorbance at 450 nm with a microplate reader. Read the absorbance within 10 minutes of adding the stop solution.

C. Calculations

This assay is competitive, therefore there is an inverse correlation between TCs concentration in the sample and the absorbance measured. The absorbance (%) should be calculated as follows:

$$Absorbance\ (\%) = \frac{A}{A_0} \times 100$$

where:

A	Average absorbance of standard/sample
A_0	Average absorbance of 0 ng/ml standard

Plot the Absorbance (%) on the y-axis, and the log concentration (ng/ml) on the x axis. Apply a best fit trendline through the standard points. Use this graph calculate sample concentrations based on their OD values. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Note: If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.

C. Precautions

Bring all reagents to room temperature prior to use.

1. Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results. It is recommended to store samples to be used within 5 days at 4°C, within 1 month at -20°C and within 2 months at -80°C.
2. Do not allow the wells to dry fully during the washing procedure. Ensure the Wash Buffer is thoroughly mixed, and the wells are washed completely.
3. Do not use the Substrate Reagent if it has begun to turn blue without being added to the wells.
4. Do not use any reagents that are expired, or use reagents from other kits with this assay.
5. The Stop Solution is caustic. Avoid contact with the skin or eyes, and thoroughly wash any spilled solution with water.
6. It is recommended that the same operator perform the experiment throughout, to avoid inconsistencies between operator procedures.