Tetracycline (TCs) ELISA Kit

Catalog No.: abx364845 **Revision date:** 29-Jan-19

Size: 96T

Storage: Store at 4°C for up to 6 months. For long term storage, the ELISA plate, Standards and Biotin conjugated antibody can be stored at -20°C.

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

Sensitivity: 0.3 ppb (ng/ml)

Detection Limit: Tissue/Liver/Egg – 1.8 ppb, Honey – 12 ppb, Urine – 3 ppb

Cross-reactivity: Tetracyclines – 100%, Chlortetracycline – 340%, Terramycin – 51%, Deoxytetracycline – 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 indirect-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. Standard: 1 ml each of:
   0 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb, 24.3 ppb, 1.0 ppm
3. HRP conjugate reagent: 11 ml
4. Antibody solution: 5.5 ml
5. Substrate reagent A: 6 ml
6. Substrate reagent B: 6 ml
7. Stop solution: 6 ml
8. Wash buffer (20X): 40 ml
9. Reconstitution buffer (5X): 50 ml

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. Trichloroacetic acid
2. Methanol
3. Deionized water

Protocol

A. Preparation of sample and reagents

1. Preparation of Sample pretreatment solutions

- **Solution 1 – 1% Trichloroacetic Acid Solution**
  Dissolve 1 g of trichloroacetic acid in 100 ml deionized water. Mix thoroughly.

- **Solution 2 – Reconstitution Solution (1X)**
  Dilute the 5× Reconstitution Buffer 5-fold with deionized water (i.e. dilute 10 ml 5× Reconstitution buffer in 40 ml deionized water) to make the 1× Reconstitution Buffer solution. The 1× solution can be stored at 4°C for up to one month.

2. Sample pretreatment

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:** Homogenize sample with a homogenizer. Weigh 2 ± 0.05 g of homogenate and add to a 50 ml tube. Add 6 ml of 1% Trichloroacetic Acid (Solution 1) and mix for 2 min on an orbital shaker. Centrifuge at 4000 RPM for 10 min at room temperature. Collect 1 ml of supernatant and add 1 ml of methanol. Mix for 1 min on an orbital shaker, then centrifuge at 4000 RPM for 10 min at room temperature. Collect 1 ml of supernatant (upper organic layer) and dry at 50-60°C with nitrogen or air. Dissolve the residue with 1 ml of 1× Reconstitution solution (Solution 2) and mix fully. Take 50 µl for detection and analysis.
  Note: Sample dilution factor: 6, minimum detection dose: 1.8 ppb.

- **Honey:** Weigh 1 ± 0.05 g of honey and add to a 50 ml tube. Add 2 ml of 1% Trichloroacetic Acid (Solution 1) and mix for 2 min on an orbital shaker. Add 0.1 ml of supernatant to 1.9 ml of 1× Reconstitution solution (Solution 2) and mix for 30 seconds. Take 50 µl for detection and analysis.
  Note: Sample dilution factor: 40, minimum detection dose: 12 ppb.

- **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. Dilute the clear urine 10-fold with 1× Reconstitution solution (Solution 2). Take 50 µl for detection and analysis.
  Note: Sample dilution factor: 10, minimum detection dose: 3 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. Wash buffer

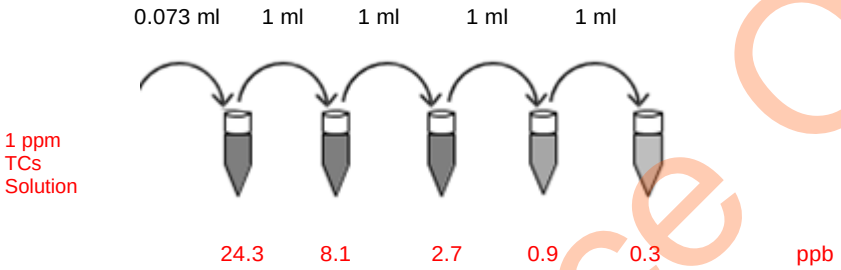
Dilute the concentrated Wash buffer 20-fold (1/20) with distilled water (i.e. add 40 ml of concentrated wash buffer into 760 ml of distilled water).

4. 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 (Solution 2) to the 0 ppb standard vial to prepare the working 0 ppb standard solution. Add 2 ml of Reconstitution Solution to each of the 0.3 ppb, 0.9 ppb, 2.7 ppb and 8.1 ppb standard vials. Add 2.93 ml of Reconstitution Solution to the 24.3 ppb standard vial.

Take 73 µl of 1.0 ppm standard and add to the 24.3 ppb standard vial, then mix thoroughly to prepare the working 24.3 ppb standard solution. Add 1 ml of working 24.3 ppb standard solution to the 8.1 ppb standard vial, and mix thoroughly to prepare the working 8.1 ppb standard solution. Add 1 ml of the working 8.1 ppb standard solution to the 2.7 ppb vial, and mix thoroughly. Add 1 ml of the working 2.7 ppb standard solution to the 0.9 ppb vial, and mix thoroughly to prepare the working 0.9 ppb standard solution. Add 1 ml of working 0.9 ppb standard solution to the 0.3 ppb vial, and mix thoroughly to prepare the working 0.3 ppb standard solution.



B. Assay Procedure

Bring all reagents to room temperature prior to use.

1. Set standard, test 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 prepared standards solutions into the standard wells.
3. Add 50 µl of PBS into the control (zero) well.
4. Add 50 µl of appropriately diluted sample into test sample wells.
5. Immediately add 50 µl of Antibody solution. Add the solution at the bottom of each well without touching the side wall.
6. Cover the plate with the plate sealer. Tap the plate gently to ensure thorough mixing. Incubate at 37°C for 30 minutes.
7. 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 250 µl of wash buffer to each well and soak for at least 1 min. Discard the contents and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure for a total of five times.

Note: For automated washing, discard the solution in all wells and wash five times overfilling the wells with Wash buffer. After the final wash invert the plate and tap on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 min.

8. Add 50 µl of HRP conjugate reagent into each well Add the solution at the bottom of each well without touching the side wall.
9. Cover the plate with the plate sealer. Tap the plate gently to ensure thorough mixing. Incubate in the dark at 37°C for 30 minutes.
10. Repeat the wash step as described in step 7.
11. Add 50 µl of substrate solution A and 50 µl of substrate solution B into each well. Cover the plate and incubate at 37°C in dark conditions for 15-20 minutes (incubation time is for reference only, do not exceed 30 minutes). When an apparent gradient appears in the standard wells the reaction can be terminated.
12. Add 50 µl of Stop solution into each well (including the blank well). There should be a colour change to yellow. Gently mix the plate to ensure thorough mixing.

13. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

This assay is competitive, therefore there is an inverse correlation between TCs concentration in the sample and the absorbance measured. Create a graph with the log of the standard concentration (y-axis) and absorbance measured (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

1. Equilibrate all reagents to room temperature (18-25°C) prior to use and centrifuge the tubes briefly in case any contents are trapped in the lid.
2. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.
3. Wash buffer may crystallize and separate. If this happens, please warm the tube and mix gently to dissolve.
4. Avoid foaming or bubbles when mixing or reconstituting components.
5. It is recommended to assay all standards, controls and samples in duplicate or triplicate.
6. Do NOT let the plate dry out completely during the assay as this will inactivate the biological material on the plate.
7. Ensure plates are properly sealed or covered during incubation steps.
8. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
9. To avoid cross contamination do not reuse pipette tips and tubes.
10. Do not use components from a different kit or expired ones.
11. Substrate solution A and substrate solution B are easily contaminated; work under sterile conditions when handling these solutions. The substrate solutions should also be protected from light. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.