Oxytetracycline ELISA Kit

Catalog No.: abx050287 **Revision date:** 4-Sep-19

Size: 96T

Storage: Store at 4°C for up to 12 months.

Application: For quantitative detection of oxytetracycline in tissues (including liver), urine, eggs, and honey.

Sensitivity: 0.01 ppb (ng/g)

Detection Limit: Honey – 2 ppb, Urine – 0.5 ppb, Tissues – 0.4 ppb, Eggs – 0.4 ppb

Cross-reactivity: Tetracycline – 100%, Chlortetracycline – 16.7%, Deoxytetracycline – 4.2%

Introduction: Oxytetracycline (trade name Terramycin) is a member of the tetracycline class of antibiotics. Its name derives from the additional hydroxyl group on the 6th carbon. It is a broad-spectrum antibiotic that binds to the 30S ribosomal subunit and prevents aminoacyl-tRNA from binding. Oxytetracycline is often used to treat infections with spirochaetes, clostridia, chlamydia, and mycoplasma. Adverse effects include gastrointestinal disturbances, photosensitivity, allergies, and susceptibility to candidiasis. Resistance to tetracyclines is mediated by binding site mutations, conformational changes via ribosomal protection proteins, degradation or modification of the molecule, and removing the antibiotic using an efflux pump.

Principle of the Assay

This kit is based on indirect-competitive enzyme-linked immuno-sorbent assay technology. Oxytetracycline is pre-coated onto a 96-well plate. The standards and samples and a biotin conjugated antibody specific to oxytetracycline 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 oxytetracycline 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 oxytetracycline amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of oxytetracycline can be calculated.

Kit components

1. One pre-coated 96 well plate
2. Standard vials - 0.05 ppb, 0.15 ppb, 0.45 ppb, 1.35 ppb, 4.05 ppb
3. 1000 ppb standard: 1 ml
4. HRP conjugate reagent: 11 ml
5. Antibody solution: 5.5 ml
6. Substrate reagent A: 6 ml
7. Substrate reagent B: 5 ml
8. Stop solution: 6 ml
9. Wash buffer (20X): 40 ml
10. Reconstitution buffer (5X): 50 ml
11. Plate sealers: 3
12. Hermetic bag: 1

Materials Required But Not Provided

1. 25°C incubator
2. Microplate reader (450 nm)
3. High-precision pipette and sterile pipette tips
4. Automated plate washer
5. ELISA shaker
6. 50 ml centrifuge tubes
7. Absorbent filter papers
8. 100 ml and 1 L graduated cylinders
9. Nitrogen or water bath
10. Homogenizer

Reagents Required But Not Provided

1. Deionized water
2. N,N-dimethylformamide

Protocol

A. Preparation of sample and reagents

1. Preparation of reagents

- **Standard solutions**
  Add 12 µl of the standard solution and 3 ml of reconstitution buffer to the 4.05 ppb vial. Mix well, then add 1 ml of this dilution to 2 ml of reconstitution buffer in the 1.35 ppb vial. Mix well, then add 1 ml of this dilution to 2 ml of reconstitution in the 0.45 ppb vial. Repeat these steps for the 0.15 ppb and 0.05 ppb vials. To the 0.00 ppb vial add 1 ml of reconstitution buffer directly.

- **Wash buffer**
  Dilute the 20× wash buffer 20-fold with deionized water (i.e. dilute 40 ml 20× wash buffer in 760 ml deionized water) to make the 1× wash buffer solution.

- **Reconstitution buffer**
  Dilute the 5× wash buffer 2-fold with deionized water (i.e. dilute 50 ml 20× wash buffer in 200 ml deionized water) to make the 1× reconstitution buffer solution.

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.

- **Honey:** Weigh 1 g of honey and add to a 15 ml centrifuge tube. Add 2 ml of dimethylformide and vortex for 2 minutes. If sample contains precipitate (cloudiness), centrifuge at 2000 rpm g 5 min. Aspirate the supernatant and add 50 µl to 450 µl of deionized water.
  Note: Sample dilution factor: 40, minimum detection dose: 3 ppb.

- **Urine:** If sample is cloudy, centrifuge at 4000 rpm for 10 minutes and aspirate the supernatant. Dilute 1:10 with reconstitution buffer. Aliquot 50 µl for analysis.
  Note: Sample dilution factor: 10, minimum detection dose: 0.5 ppb.

- **Tissues, Egg:** Homogenise samples. Weigh 2 g of sample and add to a 15 ml centrifuge tube. Add 4 ml of dimethylformide and vortex for 5 minutes. Centrifuge at 4000 rpm for 10 min. Aspirate the supernatant and add 50 µl to 750 µl of reconstitution buffer. Vortex for 30 seconds, and aliquot 50 µl for analysis.
  Note: Sample dilution factor: 8, minimum detection dose: 0.4 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.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

1. Set standard and sample 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. Add the solution at the bottom of each well without touching the side wall.
3. Add 50 µl of prepared sample into the sample wells.
4. Add 50 µl of antibody solution to each well.
5. Cover the plate with the plate sealer. Tap the plate gently to ensure thorough mixing. Incubate at 37°C for 30 minutes in dark conditions.
6. Remove the cover, and discard the solution without touching the side walls. Blot the plate on an absorbent material. Add 350 µl of wash buffer to each well and soak for at least 30 seconds. Discard the contents and blot the plate on 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 minute.

7. Add 100 µl of HRP conjugate reagent into each well. Add the solution at the bottom of each well without touching the side walls.
8. 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.
9. Repeat the wash step as described in step 6.
10. Add 50 µl of substrate solution A and 50 µl of substrate solution B into each well. Tap the plate gently to ensure thorough mixing. 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.
11. Add 50 µl of Stop solution into each well (including the blank well). There should be a colour change to yellow. Gently tap the plate to ensure thorough mixing.
12. 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.
13. OPTIONAL: measure the absorbance at 630 nm and subtract these values from the 450 nm values for analysis.

This assay is competitive, therefore there is an inverse correlation between oxytetracycline 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 replace the residual solution back into the vial.