Instructions for Use

Version: 1.0.1

Tetracycline (TCs) ELISA Kit

Catalog No.:abx365000

Size: 96T

Storage: Store at 4°C.

Application: For quantitative detection of Tetracycline in muscle, egg and milk samples.

Sensitivity: 0.15 ppb (ng/ml)

Detection Limit: Muscle (method 1), Eggs - 3 ppb; Muscle (method 2), Liver – 100 ppb; Feed – 200 ppb; Raw Milk, Finished Milk – 10 ppb

Cross-Reactivity: Tetracycline – 100%; Oxytetracycline – 46.4%; Chlortetracycline – 15.4%; Doxycycline – 8.9%

Sample Recovery Rate: 90% ± 30%

Introduction: Tetracyclines are broad-spectrum antibiotics, and are the most common veterinary drugs. In addition to therapeutic use, in many other countries, tetracycline is usually added to livestock feed in sub-therapeutic doses as a growth promoter for pigs, poultry, and aquaculture. Excessive use leads to the emergence of antibiotic resistance and allergic reactions. The COMMISSION REGULATION (EU) has set the maximum residue limit (MRL) in muscle and milk to 100ppb.

Principle of the Assay

This kit is based on indirect-competitive enzyme-linked immuno-sorbent assay technology. Tetracycline is pre-coated onto a 96-well plate. The standards and samples and a biotin conjugated antibody specific to Tetracycline 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 Tetracycline will produce a blue color product that changes into yellow after adding the stop solution. The intensity of the yellow color is inversely proportional to the Tetracycline amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of Tetracycline can be calculated.

Kit components

1. One pre-coated 96 well plate
2. Standard vials 0 ppb, 0.15 ppb, 0.45 ppb, 1.35 ppb, 4.05 ppb, 12.5 ppb: 1 ml each
3. Wash Buffer (20X): 25 ml
4. Sample Solution 1 (20X): 50 ml
5. Detection Reagent A: 7 ml
6. Detection Reagent B: 7 ml
7. Substrate Reagent A: 6 ml
8. Substrate Reagent B: 6 ml
9. Stop Solution: 6 ml
10. Plate Sealer: 3
11. Hermetic Bag: 1

Materials Required But Not Provided

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

Reagents Required But Not Provided

1. Deionized water
2. Trichloroacetic acid

Protocol

A. Preparation of sample and reagents

1. Preparation of reagents

- **Wash Buffer Solution**
  Dilute the 20X Wash Buffer 20-fold with deionized water (e.g. dilute 25 ml 20X wash buffer in 475 ml deionized water) to make the 1X Wash Buffer Solution.

- **Sample Solution**
  Dilute the 20X Sample Solution 20-fold with deionized water (i.e. dilute 50 ml 20X Sample Solution in 950 ml deionized water) to make the 1X Sample Solution 1.

- **Trichloroacetic acid solution**
  Dissolve 1g of Trichloroacetic acid in 100ml deionized water. Mix fully.

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.

- **Muscle (method 1):** Remove fat from sample and homogenize. Add 1g into a 50 ml centrifuge tube. For duck, add 9 ml of deionized water; for other muscle, add 9 ml of sample solution. Vortex for 1 minute, then centrifuge at 4000 x g for 10 minutes at room temperature.
  Note: Sample dilution factor: 10, minimum detection dose: 3 ppb.

- **Muscle (method 2):** Remove fat from sample and homogenize. Add 1g into a 50 ml centrifuge tube. Add 4 ml of Trichloroacetic acid solution, then centrifuge at 4000 x g for 5 minutes at room temperature. Take 40 µl of the supernatant from the upper layer and add 1560 µl of Sample Solution and vortex for 30 seconds. Take 50 µl for analysis.
  Note: Sample dilution factor: 200, minimum detection dose: 100 ppb.

- **Raw and Finished Milk:** Add 100 µl of fresh sample to a 2 ml centrifuge tube, then add 900 µl of Sample Solution and mix fully. Take 50 µl for analysis.
  Note: Sample dilution factor: 10, minimum detection dose: 10 ppb.

- **Egg:** Weigh 1g of homogenized sample into a 50 ml centrifuge tube, then add 5 ml deionized water. Vortex for 1 minute. Centrifuge at 4000 x g for 10 minutes at room temperature. Take 1 ml of the supernatant from the upper layer and add 1 ml of Sample Solution. Vortex for 30 seconds, then centrifuge at 4000 x g for 5 minutes at room temperature. Take 50 µl of the supernatant for analysis.
  Note: Sample dilution factor: 12, minimum detection dose: 3 ppb.

- **Feed:** Weigh 1g of comminuted sample into a 50 ml centrifuge tube, then add 5 ml of Trichloroacetic acid. Vortex for 2 minutes, then centrifuge at 4000 x g for 10 minutes at room temperature. Take 40 µl of the supernatant and add 1560 µl of sample solution. Vortex for 30 seconds, then take 50 µl for analysis.
  Note: Sample dilution factor: 200, minimum detection dose: 200 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 samples slowly to room temperature. 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 Detection Reagent A to each well.
5. Cover the plate with the plate sealer. Tap the plate gently to ensure thorough mixing. Incubate at 25°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 260 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 Detection Reagent B 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 25°C for 30 minutes.
9. Repeat the wash step as described in step 6.
10. Add 50 µl of Substrate Reagent A and 50 µl of Substrate Reagent B into each well. Tap the plate gently to ensure thorough mixing. Cover the plate and incubate at 25°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 color 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 Tetracycline 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 Reagent A and Substrate Reagent 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.