Oxytetracycline (OTC) ELISA Kit

Catalog No.: abx365129

Size: 96 tests

Storage: Store at 4°C.

Application: The quantitative detection of Oxytetracycline in Raw Milk, Eggs, Muscle tissue, and Feed.

Detection Limits: Raw Milk - 6 ng/ml; Egg - 6 ng/ml; Muscle - 6 ng/ml; Feed - 400 ng/ml

Cross-Reactivity: Chlortetracycline – 100 %; Tetracycline – 100 %; Doxycycline – 18.5 %

Sample Recovery Rate: 90 ± 30 %

Principle of the Assay: This kit is based on competitive enzyme-linked immuno-sorbent assay technology. Oxytetracycline antigen is precoated onto a 96-well plate. The standards, samples, Avidin conjugated to Horseradish Peroxidase, and a biotin-conjugated antibody specific to Oxytetracycline are added to the wells and incubated. Unbound conjugates are removed in a wash step. After TMB substrate solution is added only wells that contain bound Oxytetracycline antibody 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 Oxytetracycline amount present in the sample. 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. 96-well microplate
2. Standards (1 ml each): 0 ng/ml, 0.3 ng/ml, 0.9 ng/ml, 2.7 ng/ml, 8.1 ng/ml, 24.3 ng/ml
3. Detection Reagent A: 7 ml
4. Detection Reagent B: 7 ml
5. Sample Diluent (20X): 50 ml
6. TMB Substrate A: 6 ml
7. TMB Substrate B: 6 ml
8. Stop Solution: 6 ml
9. Wash Buffer (20X): 25 ml
10. Plate sealer: 3
11. Hermetic bag: 1

Materials Required But Not Provided

1. Incubator
2. Microplate reader (450 nm)
3. High-precision pipette and sterile pipette tips
4. Automated plate washer
5. ELISA shaker
6. Centrifuge
7. 5 ml centrifuge tubes
8. 50 ml centrifuge tubes
9. Absorbent filter papers
10. Vortex Mixer
11. Homogenizer

Reagents Required But Not Provided

1. Deionized water
2. Trichloroacetic acid ($C_2HCl_3O_2$)
3. Sodium Nitroprusside Dihydrate ($Na_2Fe(CN)_5NO{\bullet}2H_2O$)
4. Zinc Sulfate Heptahydrate ($ZnSO_4{\bullet}7H_2O$)

Protocol

Bring all reagents and samples to room temperature before use.

A. Preparation of Samples and Reagents

1. Reagents

- **Solution 1 – 0.36 M ($Na_2Fe(CN)_5NO•2H_2O$ Solution** (for **Raw Milk** samples)

Dissolve 0.54 g NaOH in 4.7 ml deionized water. Mix thoroughly.

- **Solution 2 – 1 M $ZnSO_4•7H_2O$ Solution** (for **Raw Milk** samples)

Dissolve 1.44 g $ZnSO_4•7H_2O$ in 4.32 ml deionized water. Mix thoroughly.

- **Solution 3 – 1 % Trichloroacetic acid Solution** (for **Feed** samples)

Dissolve 1 g Trichloroacetic acid in 100 ml deionized water. Mix Thoroughly.

- **1X Sample Diluent** (for **Feed, Egg,** and **Muscle** samples)

Dilute the provided 20X Sample Diluent 20-fold with deionized water (i.e. in a 1:19 ratio of 20X Reconstitution Buffer to deionized water).

- **1X Wash Buffer**

Dilute the provided 20X Wash Buffer 20-fold with deionized water (i.e. in a 1:19 ratio of 20X Wash Buffer to deionized water).

2. Sample Pretreatment

- **Raw Milk:** Add 3 ml of Raw Milk sample into a 5 ml centrifuge tube, then centrifuge at 4000 × g for 10 minutes at 4°C. Remove the upper fat layer and transfer 2 ml of the lower layer to a fresh tube. Add 50 µl of 0.36 M ($Na_2Fe(CN)_5NO•2H_2O$ Solution and vortex mix for 90 seconds. Add 50 µl of 1 M $ZnSO_4•7H_2O$ Solution and vortex mix for 60 seconds. Centrifuge at 3000 × g for 10 minutes, then transfer 50 µl of supernatant to a fresh tube. Dilute the supernatant with 450 µl of Deionized Water and collect 50 µl for analysis.

Note: Sample dilution factor: 10; Detection limit: 6 ng/ml.

- **Feed:** Homogenize the Feed sample. Weigh 1 g of Feed homogenate into a centrifuge tube and add 5 ml of 1 % Trichloroacetic acid Solution. Vortex for 5 minutes, then centrifuge at 4000 × g for 10 minutes. Transfer 40 µl of supernatant to a fresh tube, then dilute with 1.56 ml 1X Sample Diluent and vortex mix for 30 seconds. Collect 50 µl for analysis.

Note: Sample dilution factor: 200; Detection limit: 400 ng/ml.

- **Eggs:** Homogenize the Egg sample. Weigh 1 g of homogenate into a 50 ml centrifuge tube, then add 5 ml of Deionized Water and vortex for 2 minutes. Centrifuge at 4000 × g for 10 minutes, then transfer 1 ml of supernatant to a fresh tube. Add 1 ml of 1X Sample Diluent and vortex mix for 30 seconds. Centrifuge at 4000 × g for 5 minutes, then collect 50 µl of supernatant for analysis.

Note: Sample dilution factor: 12; Detection limit: 6 ng/ml.

- **Muscle:** Remove as much fat as possible from the sample, then homogenize and mix fully. Weigh 1 g of homogenate into a 50 ml centrifuge tube, then add 9 ml of 1X Sample Diluent and vortex mix for 1 minute. Centrifuge at 4000 × g for 10 minutes, then collect 50 µl of supernatant for analysis.

Note: Sample dilution factor: 10; Detection limit: 6 ng/ml.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

1. Number the sample and ordered standard wells, and record their positions. *All samples and standards should be tested in duplicate.*
2. Add 50 µl of standard or sample into the respective standard and sample wells.
3. Add 50 µl Detection Reagent B to each well,
4. Add 50 µl Detection Reagent A to each well.
5. Cover the plate with a plate sealer, shake gently for 10 seconds to mix, and incubate at 25°C for 30 minutes in the dark.
6. Carefully remove the plate sealer, and remove the liquid in each well. Immediately add 260 µl 1X Wash Buffer and wash. Repeat this wash procedure for a total of 5 times, with 30 second intervals. Invert the plate and pat onto absorbent towels. Prick any bubbles remaining in the wells with a clean pipette.
7. Add 50 µl of TMB Substrate A to each well.
8. Add 50 µl of TMB Substrate B to each well. Shake gently for 10 seconds to ensure thorough mixing, and incubate at 25°C for 15 minutes in the dark.
9. Add 50 µl of Stop Solution to each well, and shake gently to ensure thorough mixing.
10. Within 5 minutes of introducing the Stop Solution, determine the optical density of each well with a microplate reader at 450 nm.

C. Analysis of Results

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 on the y-axis, and absorbance measured on the x-axis (a semi-logarithmic plot). Apply a best fit trendline through the standard points. Use this graph to calculate sample concentrations based on their OD values.

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

D. 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 TMB Substrate 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.

E. Technical Support

For troubleshooting, frequently asked questions, and assistance, please visit: https://www.abbexa.com/scientific-support/troubleshooting-and-faqs/elisa-kit-scientific-support or email us at support@abbexa.com.