Tetracycline ELISA Kit

Enzyme Immunoassay for the quantification of tetracycline in food.

Catalog number: ARG81035

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Tetracyclines have a broad spectrum of antibiotic action. Originally, they possessed some level of bacteriostatic activity against almost all medically relevant aerobic and anaerobic bacterial genera, both Gram-positive and Gram-negative, with a few exceptions, such as Pseudomonas aeruginosa and Proteus spp., which display intrinsic resistance.

The tetracyclines also have activity against certain eukaryotic parasites, including those responsible for diseases such as dysentery caused by an amoeba, malaria (a plasmodium), and balantidiasis (a ciliate).

Common side effects include vomiting, diarrhea, rash, and loss of appetite. Other side effects include poor tooth development if used by children less than eight years of age, kidney problems, and sunburning easily. Use during pregnancy may harm the baby. Tetracycline is in the tetracyclines family of medications. It works by blocking the ability of bacteria to make proteins. [Provide by Wikipedia: Tetracycline]

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. A capture antibody specific for tetracycline has been pre-coated onto a microtiter plate. Tetracycline containing samples or standards and a tetracycline-peroxidase conjugate are given into the wells of the microtiter plate. The tetracycline conjugate competes with the tetracycline of the samples / standards for the limited number of antibody sites. After incubation, the wells are washed with wash buffer to remove unbound material. Then the TMB substrate is added to the wells and color develops in proportion to the amount of tetracycline bound in the initial step. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450nm.The concentration of tetracycline in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C
100X Standards (Tetracycline 0, 0.04, 0.1, 0.4, 1, and 4 ng/mL)	1 mL each, 6 vials	4°C
10X Diluent Buffer	60 mL X 2 (dyed red)	4°C
Tetracycline Conjugate (Tetracycline-peroxidase)	3 vials (dyed red, lyophilized)	4°C
10X Wash Buffer	60 mL	4°C
TMB substrate	15 mL (ready to use)	4°C (Protect from light)
STOP solution	15 mL (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- Hexan
- Volumetric flask
- Water bath
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- Stored at 2-8°C the diluted standards are stable for at least 12 hours.
- The provided conjugate is freeze dried and has to be reconstituted before the test. For dissolution add 2.5 mL of distilled water per vial and shake well for 5 minutes. The re-dissolved HRP-Conjugate antibody can be stored frozen at -20°C for at least 1 month. Repeated freezing and thawing should be avoided.
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash buffer and 10X Diluent buffer, warm to 37°C until the crystals are completely dissolved.

- Due to high risk of cross-contamination all applied instruments like applicator, mortar, glass vials etc. have to be cleaned thoroughly before and after each sample.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, do NOT use it.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Use a new adhesive plate cover for each incubation step.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

Milk or milk powder containing samples are not suitable for testing by ARG81035 tetracycline ELISA kit

The following sample preparation should be applied for liquid samples:

1. Defat sample if applicable. Therefore the liquid sampe has to be centrifuged for 15 min at 4°C and at least 2000 g. Afterward the upper fat layer should be removed.
2. Dilute 1 mL of previously liquid sample in 9 mL of Diluent buffer. Afterwards the solution is shaken for 5 mins at room temperature. The process is continued at point 3 of solid sample extraction process.

The following sample preparation should be applied for solid samples:

1. To maximize homogeneity and representativeness of the sample drawing, a minimum of 5 g sample should be pulverized finely in a mortar, impact mill etc.
2. 1 g of the homogenized mixture is suspended in 10 mL of 1X Diluent buffer. Afterwards the suspension is shaken for 10 mins at 40°C (Cheese, Honey, and Meat) or at 60°C (Nonfat dry milk).
3. The samples are centrifuged for 15 minutes with at least 2000 g. For a better separation of fat the centrifuge should be cooled to 4°C if applicable. If it is not possible to separate the supernatant from the precipitate completely, the suspension should be filtrated if necessary.
4. Further treatment:

   a) shrimps samples:

      Dilute 100 µL of particle-free solution with 100 µL of 1X Diluent buffer. 100 µL of the diluted extract are applied per well.

Dilution factor = 20

b) Meat samples:

Extract 1 mL of particle-free solution with 2 mL Hexan and discard the Hexan layer. Dilute 100 µL of extract with 100 µL of 1X Diluent buffer. 100 µL of the diluted extract are applied per well.

Dilution factor = 20

c) Cheese samples:

Extract 1 mL of particle-free solution with 2 mL Hexan and discard the Hexan layer. Dilute 100 µL of extract with 400 µL of 1X Diluent buffer. 100 µL of the diluted extract are applied per well.

Dilution factor = 50

d) Honey samples:

Dilute 100 µL of particle-free solution with 400 µL of 1X Diluent buffer. 100 µL of the diluted extract are applied per well.

Dilution factor = 50

In case of too high concentrated samples, the sample extracts have to be further diluted with 1X Diluent buffer. The additional dilution factor has to be accounted for when calculating the results.

REAGENT PREPARATION

- **1X Wash Buffer**: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer. (E.g., add 50 ml of 10X Wash buffer into 450 ml of distilled water to a final volume of 500 ml) The 1X Washing Buffer is stable for up to 4 weeks when stored at 2-8°C.
- **Tetracycline Conjugate**: 3 vials with 2.5 mL each, dyed red, lyophilized. The provided tetracycline conjugate is freeze dried and has to be reconstituted before the test. For dissolution add 2.5 mL of distilled water per vial and shake well for 5 minutes. The re-dissolved tetracycline conjugate can be stored frozen at-20 °C for at least 1 month. Repeated freezing and thawing should be avoided.
- **Diluent Buffer**: Dilute 10X Diluent buffer (dyed red) into distilled water to yield 1X Diluent buffer. (E.g., add 50 ml of 10X Diluent buffer into 450 ml of distilled water to a final volume of 500 ml) The 1X Diluent Buffer is stable for up to one week when stored at 2-8°C.
- **Standards (Tetracycline):** 6 vials with 1 mL each as 100x concentrate, dyed red. Dilute 20 µL of standard with 1980 µL 1X Diluent buffer to achieve the following concentrations (0; 0.04; 0.1; 0.4; 1; 4 ng/mL).

ASSAY PROCEDURE

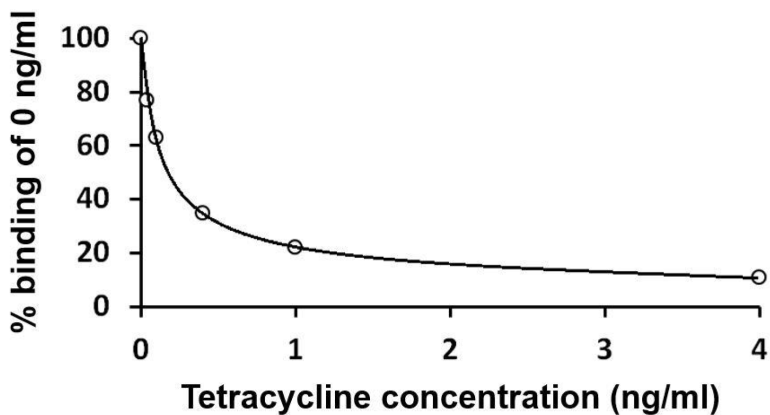
All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards, samples and controls should be assayed in duplicates.

1. Add **100 µL** of **sample or standard** to the Antibody-coated microplate.
2. Add **50 µL** of the **re-dissolved Tetracycline conjugate** to each well. Incubate at **RT** for **1 hour** on a microplate shaker.
3. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with **1× Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µl** of **TMB Substrate** to each well. Incubate for **20 minutes** at **RT** in the dark.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
5. Add **100 µl** of **Stop Solution** to each well. The color of the solution should change from blue to yellow.
6. Read the OD with a microplate reader at **450nm** immediately. (optional: read at ≥ 620 nm as reference wavelength) It is recommended reading the absorbance **within 30 minutes** after adding the stop solution.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Tetracycline ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Us the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for the detail. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

QUALITY ASSURANCE

Sensitivity

The limit of detection (LOD) of the Tetracycline test is 0.024 ng/mL.

The limit of quantification (LOQ) of the Tetracycline test is 0.072 ng/mL.

Validation experiments with common matrices resulted in the following LODs and LOQs [ppb].

Matrix	LOD	LOQ
Meat	0.5	1.4
Cheese	1.4	3.2
Shrimps	0.5	1.1
Honey	2.2	3.3

Specificity

The antibody is directed specifically against the Tetracycline.

Cross reactivity values have been calculated on a weight/weight basis.

Tetracycline	100%
4-Epitetracycline	111%
Rolitetracycline	82%
Chlortetracycline	42%
Doxycycline	41%
Demeclocycline	37%
Oxytetracycline	34%
4-Epioxytetracycline	34%
4-Epichlortetracycline	11%
Methacycline	9%
Minocycline	1%

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 4% and inter-assay precision was 13%.

Recovery

Meat	87%
Cheese	82%
Shrimps	89%
Honey	103%