Tetracycline ELISA Kit

(One-Step Procedure)

Enzyme-Linked Immunosorbent Assay for Detection of Tetracycline

Cat. No.: ELISA-TET-001

96 TESTS

For *in vitro* use only

Please read this package insert completely before using this product

Background

Tetracycline (TET) is a broad-spectrum polykenetic antibiotic derived from *Streptomyces* spp. and used for the treatment and prevention of many bacterial infections. Due to its low cost, broad spectrum activity and low toxicity profile it is widely used as a feed additive for food-producing animals and in aquaculture; however, over use can lead to antibiotic resistance. The monitoring of food products for tetracycline is necessary to determine that these compounds are not misused and do not present a danger to human or animal health.

Intended Use

The Biopanda TET ELISA kit is a competitive immunoassay to quantitatively detect the presence of TET in seafood, meat, milk, milk powder, honey and egg samples.

Detection Limit – Seafood Sample: 1.2 ppb
Fish Sample: 1.2 ppb
Pork Sample: 1.2 ppb
Milk Sample: 1.2 ppb
Milk Powder Sample: 6 ppb
Honey Sample: 5 ppb
Egg Sample: 3 ppb

Recovery Rate – Seafood Sample: 75-125%
Fish Sample: 85-130%
Pork Sample: 85-115%
Milk Sample: 70-110%
Milk Powder Sample: 63-123%
Honey Sample: 70-130%
Egg Sample: 75-120%

Specificity (Cross-Reactivity) – Tetracycline: 100%
Oxytetracycline: 50%
Chlortetracycline: 50%
Doxycycline: 20%

Principle

This test kit is based on a competitive enzyme-linked immunosorbent assay (ELISA) for the detection of TET. An unknown amount of TET present in the sample and the fixed amount of TET antigens pre-coated onto the wells of microtiter plate/strips compete for the anti-TET antibodies, which in turn are detected with enzyme conjugate. After incubation, the wells are washed and the bound enzyme is visualised by adding TMB solution. Any coloured product is measured at 450 nm after adding stop solution. The absorbance value of the developed colour is inversely proportional to the amount of TET in the sample. The quantity of TET in the test sample can be calculated using the standard curve constructed from the standards, and corrected for the sample dilution.

Storage and Stability

- The kit should be stored at 2–8°C. Do not freeze.
- Unused test wells should be sealed and stored at 2–8°C.
- This kit is valid until the expiration date printed on the label.

Kit Components Supplied

Item	Description
1	1 × Pre-coated microtiter plate (12 × 8 microwells)
2	1 × Antibody solution (7 ml)
3	1 × Enzyme conjugate (7 ml)
4	1 × Wash buffer concentrate (20×, 30 ml)
5	6 × TET standard concentrates (50×, 0.5 ml each)
6	1 × High Concentrate of TET standard (0.5 ml)*
7	1 × Assay Diluent concentrate (5×, 10 ml)
8	1 x Sample extraction buffer concentrate (20x, 30 ml)
9	1 × TMB solution (12 ml)
10	1 × Stop solution (12 ml)
11	1 × Package Insert
12	1× Microplate sealer

**** This component is optional and only for the user to check the recovery rate of tetracycline.***

Materials / Equipment Required but not Included with Kit

- ELISA Microtiter plate reader equipped with 450/630 nm filters
- Multichannel micropipette
- 10-100 , 100 -1000 µl micropipette tips
- Microplate washer or squeeze bottle
- Centrifuge
- Vortexer
- Centrifugal tubes
- Deionised water (ddH_2O)
- Absorbent paper or paper towel

Precautions

- Please carefully read the instructions before use.
- Reagents should be brought to room temperature (RT, 20-25°C) prior to use.
- Do not use reagents after the expiration date. Do not use reagents from other kits with different lot numbers.
- Avoid contact of skin and mucous membranes with reagents and sample extraction. If exposure should occur, immediately flush with water.
- Please wear protective gloves when using the kit. Consider all materials that are exposed to standards or samples to be contaminated.
- Use different tips when pipetting different reagents and samples.
- Keep the stop solution away from skin and eyes.

Preparation of Working Solutions

- **Assay Diluent:** Dilute the assay diluent concentrate (5x) 5-fold with deionised water (e.g. 1 ml assay diluent concentrate with 4 ml ddH_2O).
- **Sample Extraction Buffer:** Dilute the sample extraction buffer concentrate (20x) 20-fold with deionised water (e.g. 1 ml assay diluent concentrate with 19 ml ddH_2O).
- **Wash Buffer:** Dilute the wash buffer concentrate (2x) 20-fold with deionised water (e.g. 10 ml wash buffer concentrate with 190 ml ddH_2O)

Note: Wash buffer concentrate and sample extraction buffer concentrate may form crystals at low temperature. Ensure that the crystals completely re-dissolve before dilution (By placing into a 37°C incubator or water bath if necessary).

Sample Preparations

Seafood, Fish & Pork (Dilution Factor: 12)

- Weigh 1 g of homogenised sample into a 15 ml centrifugal tube, add 5 ml of diluted sample extraction buffer and mix thoroughly for 5 min.
- Centrifuge the sample at 4000 x g for 10 min.
- Transfer 500 µl of the supernatant to another test tube.
- Add 500 µl of diluted assay diluent to the supernatant and mix thoroughly for 10 seconds.
- The sample solution is ready for testing.

Milk (Dilution Factor: 12)

- Measure 0.5 ml of milk into a 15 ml centrifugal tube, add 2.5 ml of diluted sample extraction buffer and mix thoroughly for 2 min.
- Centrifuge the sample at 8,000 x g for 15 min.
- Discard the top layer of fat and centrifuge the sample at 8,000 x g for 15 min.
- Transfer 500 µl of the supernatant to another test tube.

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- Add 500 µl of diluted assay diluent to the supernatant and mix thoroughly for 10 seconds.
- The sample solution is ready for testing.

Milk Powder (Dilution Factor: 60)

- Weigh 0.4 g of milk powder into a 15 ml centrifugal tube, add 2 ml of diluted sample extraction buffer and mix thoroughly for 2 min.
- Centrifuge the sample at 8,000 x g for 15 min.
- Transfer 600 µl of liquid below the lipid layer to another test tube.
- Centrifuge this sample at 8,000 x g for 15 min.
- Transfer 50 µl of the supernatant to another test tube.
- Add 450 µl of diluted assay diluent to the supernatant and mix thoroughly for 10 seconds.
- The sample solution is ready for testing.

Honey (Dilution Factor: 50)

- Weigh 0.2 g of honey sample into a 15 ml centrifugal tube and add 4.8 ml of diluted sample extraction buffer.
- Mix the sample until fully dissolved (If necessary heat the honey in a water bath at 50°C, while mixing by inversion).
- Centrifuge the sample at 8,000 x g for 10 min.
- Transfer 500 µl of the supernatant to another test tube.
- Add 500 µl of diluted assay diluent to the supernatant and mix thoroughly for 10 seconds.
- The sample solution is ready for testing.

Egg (Dilution Factor: 30)

- Weigh 1 g of homogenised egg sample into a 15 ml centrifugal tube, add 5 ml of diluted sample extraction buffer and mix thoroughly for 5 min.
- Centrifuge the sample at 8,000 x g for 15 min.
- Transfer 100 µl of the supernatant to another test tube.
- Add 400 µl of diluted assay diluent to the supernatant and mix thoroughly for 10 seconds.
- The sample solution is ready for testing.

❖ ***Samples should be tested as soon as possible after preparation.***
❖ ***The sample preparation method above is only a suggestion. The kit user can use their own methods to prepare samples.***

Test Procedure

1. Ensure all reagents are equilibrated to RT prior to use. Swirl all reagents gently before use.
2. Prepare standard solutions as follows: take 6 Eppendorf tubes and mark them 1, 2, 3, 4, 5 and 6 respectively. To each tube, add 490 µl of diluted sample extraction buffer. Then take 10 µl of each TET standard 50× concentrate (0, 5, 15, 45, 135 and 405ppb) and add into tubes 1, 2, 3, 4, 5 and 6 respectively, to make 0, 0.1, 0.3, 0.9, 2.7, 8.1 ppb standard solutions.
3. Label each strip on its end tab to help identify them should they become detached from the plate frame during the assay,
4. To every well (except the two blank wells), add 50 µl of each standard solution or sample solution per well in duplicate first. Then add 50 µl of enzyme conjugate to each well. Finally add 50 µl of antibody solution to each well.
5. To the two blank wells add 100 µl of diluted sample extraction buffer and 50 µl of enzyme conjugate. (No standards/samples or antibody solution).
6. Cover the strips with the microplate sealer and shake gently to mix for 1 minute. Incubate the plate for 30 minutes at 25°C in the dark.
7. After incubation, remove the plate sealer and wash the strips 5 times with diluted wash buffer, ensuring every well is filled. When washing is completed, tap the strips firmly on absorbent tissue to remove residual wash buffer.
8. Add 100 µl of TMB solution to each well and incubate for 15 minutes at 25°C in the dark.
9. Stop the reaction by adding 100 µl of stop solution to each well in the same order as TMB solution was added. Shake gently to mix.
10. Measure absorbance at 450 nm (with 620-630nm as a reference) within 10 minutes of stopping.

Test Validity

For the test to be valid, the mean absorbance of the zero standard (S1, 0 ppb) must be over 1.0.

Results Calculation

The unknown TET concentrations in the samples are determined from a calibration curve. Calculate the mean absorbance value of the two blank wells and subtract that from the mean absorbance values of all the other wells.

Define the mean corrected absorbance value of the standards and samples as B. Define the mean corrected absorbance of the zero standard as B_0. The relative absorbance can therefore be calculated as:

$$Relative\ absorbance\ (\%) = \frac{B}{B_0} \times 100$$

Plot the relative absorbance of the standards against the standard concentration to obtain a standard curve. Using the relative absorbance value of a sample, the concentration can be found by interpolation. Remember to multiply by the dilution factor to obtain the true TET concentration.

Interpolation can be performed by carrying out a 4-parameter logistic analysis, using a linear regression method, or point-to-point interpolation. Biopanda can provide an accompanying Excel spreadsheet calculator for this purpose.

Notes

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in this package insert and with adherence to good laboratory practice (GLP).
2. Factors that might affect the performance of the assay include proper instrument function/calibration, cleanliness of glassware, quality of distilled or deionised water, accuracy of reagent and sample pipetting, washing technique, incubation time and temperature.

A recommended plate layout is given as follows:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	T2	T2	T10	T10	T18	T18	T26	T26	T34	T34
B	S2	S2	T3	T3	T11	T11	T19	T19	T27	T27	T35	T35
C	S3	S3	T4	T4	T12	T12	T20	T20	T28	T28	T36	T36
D	S4	S4	T5	T5	T13	T13	T21	T21	T29	T29	T37	T37
E	S5	S5	T6	T6	T14	T14	T22	T22	T30	T30	T38	T38
F	S6	S6	T7	T7	T15	T15	T23	T23	T31	T31	T39	T39
G	B	B	T8	T8	T16	T16	T24	T24	T32	T32	T40	T40
H	T1	T1	T9	T9	T17	T17	T25	T25	T33	T33	T41	T41

'S' denotes the Standards in duplicate;
'B' denotes the Blank wells (see Step 4 of the Test Procedure);
'T' denotes the samples that are being tested in duplicate.

By following this recommended plate layout, the results from the microtiter plate reader can be copy & pasted directly into the accompanying spreadsheet calculator.

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