Oxytetracycline ELISA Kit

Enzyme-Linked Immunosorbent Assay for Detection of Oxytetracycline in Honey

Cat. No.: ELISA-OTC-002

96 TESTS

For *in vitro* use only

Please read this package insert completely before using this product

Background

The Tetracyclines are a group of closely related bacteriostatic antibiotics. Due to their broad spectrum activity, low toxicity profile and low cost, the Tetracyclines are used extensively in veterinary practice, and often used as feed additives for food-producing animals (including honeybees) and in aquaculture. Oxytetracycline (OTC) belongs to the Tetracyclines and is one of the most commonly used antibiotics. Biopanda have developed the OTC ELISA to detect traces of OTC in **honey**.

Intended use

The Biopanda OTC ELISA kit is designed to quantitatively detect the presence of OTC in **honey**.

Detection Limit - 6 ppb

Recovery Rate - 80-105%

Specificity - Oxytetracycline: 100%
Tetracycline: 200%
Chlortetracycline: 200%
Doxycycline: 20%

Principle

This test kit is based on a competitive enzyme-linked immunosorbent assay (ELISA) for the detection of OTC. An unknown amount of OTC present in the sample and the fixed amount of OTC antigens pre-coated onto the wells of microtiter plate/strips compete for the anti-OTC 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 OTC in the sample. The quantity of OTC 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 × OTC antibody solution (7 ml)
3	1 × Enzyme conjugate (7 ml)
4	1 × Wash buffer concentrate (20×, 30 ml)
5	5 × OTC standards concentrate (10×, 1.0 ml each)
6	1 × High Concentrate of OTC standard (1.0 ml) *
7	1 × Assay diluent concentrate (20×, 10 ml)
8	1 x Standard diluent concentrate (20×, 2 ml)
9	1 × TMB solution (12 ml)
10	1 × Stop solution (12 ml)
11	1 × Microplate sealer
12	1× Package Insert

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

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Materials/equipment required but not included with kit

- ELISA Microtiter plate reader equipped with 450/630 nm filters
- Multichannel micropipette
- 20 µl, 50 µl, 100 µl, 200 µl,1000 µl micropipette tips
- Microplate washer or squeeze bottle
- Centrifuge
- Vortexer
- Centrifugal tubes
- Deionised water (ddH_2O)
- n-Hexane

Precautions

- Please carefully read the instructions before use.
- Reagents should be brought to room temperature (RT) 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:* using deionised water (ddH_2O), make a 20-fold dilution of the Assay diluent concentrate (20×) (e.g. 1 ml Assay diluent concentrate with 19 ml ddH_2O).
- *Standard diluent:* using deionised water, make a 20-fold dilution of the Standard diluents concentrate (20×) (e.g. 100 µl Standard diluent concentrate with 1900 µl ddH_2O).
- *Wash buffer:* using deionised water, make a 20-fold dilution of the Wash buffer concentrate (20×) (e.g. 10 ml Wash buffer concentrate with 190 ml ddH_2O)

Note: The Assay diluent concentrate and Wash 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 Preparation

Method 1: - dilution factor: 16

1. Weight 0.5 g honey into a 15ml centrifuge tube, and add 1.5 ml ddH_2O.
2. If necessary, heat the honey about 10 minutes at 50ºC, while mixing by inversion until the sample is fully dissolved.
3. Centrifuge the sample at 4000 ×*g* for 10 min at RT. After centrifugation, a wax-like layer will form at the top.
4. Transfer 500 µl of the honey solution below the waxy layer to a microcentrifuge tube and centrifuge at 4000 ×*g* for 10 min.
5. Transfer 100 µl of this supernatant to another test tube containing 300 µl working concentration Assay diluent.
6. Vortex for 20 seconds.
7. The sample solution is now ready for testing.
   Note: If this method gives poor recovery, then use Method 2 below.

Method 2: - dilution factor: 16

1. Weigh 0.5 g of honey into a 15-ml centrifuge tube, and add 1.5 ml ddH_2O and 2 ml of **n-Hexane**. Close the tube cap tightly.
2. Heat the honey for about 10 minutes at 50ºC, while mixing by inversion until the sample is fully dissolved.
3. Open the tube cap to release any pressure built up during heating, then re-cap the tube. Mix on a roller for 10 minutes.

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4. Centrifuge for 10 minutes at 4,000 x *g* at RT. After centrifugation, there will be a wax-like layer between the bottom honey solution and upper n-Hexane phase.
5. With a pipette, discard as much of the upper n-Hexane layer as possible.
6. Transfer 500 µl of the honey solution below the waxy layer to a microcentrifuge tube and centrifuge at 4000 ×*g* for 10 min.
7. Transfer 100 µl of this supernatant to another test tube containing 300 µl working concentration Assay diluent.
8. Vortex for 20 seconds.
9. The sample solution is now ready for testing.

Samples should be tested as soon as possible after preparation. The methods above are only suggested methods for sample preparation.

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 5 Eppendorf tubes and mark them 1, 2, 3, 4, and 5, respectively. To each tube, add 450 µl of working concentration Standard diluent. Then take 50 µl of each OTC standard 10× concentrate (0, 4, 12, 36, and 108 ppb) and add into tubes 1, 2, 3, 4 and 5, respectively, to make 0, 0.4, 1.2, 3.6, and 10.8 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 OTC antibody solution to each well. ***(Note: this order of addition is very important.)***
5. To the two blank wells, add 100 µl of diluted Assay diluent and 50 µl of Enzyme conjugate (No standards/samples and antibody solution).
6. Cover the strips with the microplate sealer. Mix gently by shaking the plate for 1 minute then 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 the 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 the TMB solution was added. Shake gently to mix.
10. Measure absorbance at 450 nm (with 630nm as a reference) within 10 minutes of stopping.

Test validity

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

Results calculation

The unknown OTC 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 OTC 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	T3	T3	T11	T11	T19	T19	T27	T27	T35	T35
B	S2	S2	T4	T4	T12	T12	T20	T20	T28	T28	T36	T36
C	S3	S3	T5	T5	T13	T13	T21	T21	T29	T29	T37	T37
D	S4	S4	T6	T6	T14	T14	T22	T22	T30	T30	T38	T38
E	S5	S5	T7	T7	T15	T15	T23	T23	T31	T31	T39	T39
F	B	B	T8	T8	T16	T16	T24	T24	T32	T32	T40	T40
G	T1	T1	T9	T9	T17	T17	T25	T25	T33	T33	T41	T41
H	T2	T2	T10	T10	T18	T18	T26	T26	T34	T34	T42	T42

'S' denotes the Standards in duplicate;
'B' denotes the Blank wells (see Step 5 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.

Version 3
Revision date: 15/06/2017

Unit 14, Carrowreagh Business Park
Carrowreagh Road
Belfast, BT16 1QQ
United Kingdom

Tel: + 44 (0) 28 95438774
Fax: + 44 (0) 28 90486696
E-mail: info@biopanda.co.uk
Website: www.biopanda.co.uk