Tetracycline Plate Kit
(for Honey)

PN 52254B

Instructional Booklet
Read Completely Before Use.

INTENDED USE

The Abraxis Tetracycline Plate Kit is a competitive ELISA for the quantitative analysis of Tetracycline in Honey.

ASSAY PRINCIPLES

The Abraxis Tetracycline plate kit is a competitive enzyme-labeled immunoassay. Tetracycline is extracted from honey by shaking with extraction solution. The Tetracycline sample extract and calibrators are pipetted into the test wells followed by Tetracycline antibody into the test wells to initiate the reaction. During the 30 minute incubation period, Tetracycline from the sample and Tetracycline protein conjugate compete for binding to Tetracycline antibody. Following this 30 minute incubation, the contents of the well are removed and the wells are washed to remove any unbound Tetracycline and free Tetracycline antibody. After wash, the Goat anti-Rabbit HRP conjugate is added to each well and the plate is incubated for 30 min. After a second wash with wash solution, a clear substrate is then added to the wells and any bound enzyme conjugate causes the conversion to a blue color. Following a 30 minute incubation, the reaction is stopped and amount of color in each well is read. The color of unknown samples is compared to the color of the calibrators and the Tetracycline concentration of the samples is derived.

SPECIFICITY:

The Abraxis TETRACYCLINE Plate Kit can not differentiate between the various Tetracyclines, but detects their presence to differing degrees. The following table shows the relative values for the % cross reactivity versus Tetracycline. All concentrations are in parts per billion (ppb).

Compound	% CR
Tetracycline	**100%**
Rolitetracycline	**97%**
Chlorotetracycline-Hcl	**90%**
Demeclocycline-Hcl	**13%**
Oxytetracycline	**1.4%**
Minocycline	**0.7%**
Doxycycline Hyclate	**0.5%**

DETECTION LIMIT:

Honey: 2 ppb

REAGENTS AND MATERIALS PROVIDED

The kit in its original packaging can be used until the end of the month indicated on the box label when stored at 2 – 8ºC.

- Plate containing 12 test strips of 8 wells each vacuum-packed in aluminized pouch with indicating
- dessicant.

- 6 vials each containing 2 mL of Tetracycline calibrators corresponding to 0, 0.4, 1.2, 3.6, 10.8, and 32.4 µg/L (ppb) of Tetracycline. Calibrators are provided 10X concentrated and should be diluted prior to running the assay.

- 1 vial containing 22 mL Goat anti-Rabbit HRP Enzyme Conjugate.

- 1 vial containing 12 mL of Rabbit anti-Tetracycline antibody.

- 1 vial containing 12 mL of Substrate.

- 1 vial containing 12 mL of Stop Solution. (Caution! 1N HCl. Handle with care.)

- 1 vial containing 100 mL of 5X concentrated Wash Buffer. Solution needs to be diluted with deionized water (100 mL of 5X Wash buffer and 400 mL of deionized water)

- Instructions

PRECAUTIONS

1. Each reagent is optimized for use in the Abraxis Tetracycline Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Abraxis Tetracycline Plate Kits with different Lot numbers.

2. Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.

3. Do not use reagents after expiration date.

4. Reagents should be brought to room temperature, 20 – 28ºC (62 – 82ºF) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.

5. Tetracycline is antibiotics and should be treated with care.

6. The Stop Solution is 1N hydrochloric acid. Avoid contact with skin and mucous membranes. Immediately clean up any spills and wash area with copious amounts of water. If contact should occur, immediately flush with copious amounts of water.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Laboratory quality distilled or deionized water.
2. Graduated cylinder, 100 ml or larger.
3. Glassware for sample extraction and extract collection.
4. Ultrasonic bath
5. Pipet with disposable tips capable of dispensing 50 µL.
6. Multi-channel pipet; 8 channel capable of dispensing 50 and 100 µL.
7. Paper towels or equivalent absorbent material.
8. Microwell plate or strip reader with 450nm filter.
9. Timer
10. Vortex mixer
11. Wash bottle
12. 20 mM PBS, pH 5.0. [2.76 g NaH2PO4-H2O (F.W. 137.99) + 8.5 g NaCl, filling with 1 liter of laboratory grade water]
13. 10% Methanol/20 mM PBS is prepared as follows: 100 mL of methanol and 90 mL of 20 mM PBS.

ASSAY CALIBRATORS PREPARATION

The calibrators were provided in the kit are 10X concentrates. Before each assay, they should be diluted into 10% MeOH/20 mM PBS.

For example, mix 100 ul of 0.4 ppb with 900 ul of 10% MeOH/20 mM PBS in a small glass vial or glass tube to obtain 0.04 ppb working calibrator. All the 10X calibrators from 0 ppb to 32.4 ppb should be diluted the same way before assay.

HONEY SAMPLE PREPARATION

1. Weigh 1 gram of honey in a screw cap glass bottle (60-80 ml size)
2. Add 49 ml of 20 mM PBS (1ml sample + 49 ml buffer, 1:50 dilution)
3. Put the sample bottle in an ultrasonic water bath for 5 min.
4. Mix vigorously for 2 min.
5. Before transferring 100 ul for the assay, invert the sample bottle several times to mix.

TEST PROCEDURE (Note: Running calibrators and samples in duplicate will improve assay precision and accuracy.)

1. Allow reagents and sample extracts to reach room temperature prior to running the test. Prepare the wash solution by mixing 100 ml of 5X wash buffer concentrate with 400 ml of lab grade water.

2. Place the appropriate number of test wells and into a microwell holder. Be sure to re-seal unused wells in the zip-lock bag with dessicant.

3. Using a pipet with disposable tips, add **100 µL of calibrators and samples** to the appropriate test wells. Be sure to use a clean pipet tip for each.

4. Dispense **100 µL of Antibody Solution** into each test well.

5. Shake the plate gently for 30 seconds and incubate the test wells for **30 minutes**.

7. Dump the contents of the wells into an appropriate waste container. Add 250 uL of diluted (1X) wash buffer and dump. Repeat 3X for a total of four washes.

8. Following the last wash tap the inverted wells onto absorbent paper to remove the last of the wash solution.

9. Add **200 ul of Goat anti-Rabbit HRP conjugate to each well.**

10. Incubate for **30 min.**

11. Repeat Step 7 and 8

12. Dispense **100 µL of Substrate** into each well.

13. Incubate the wells for **30 minutes**.

14. Dispense **100 µL of Stop Solution** into each test well.

15. Read and record the absorbance of the wells at 450nm using a strip or plate reader.

RESULTS INTERPRETATION

1. Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrator wells: Sample containing less color than a calibrator well will have a concentration of Tetracycline greater than the concentration of the calibrator. Samples containing more color than a calibrator well have a concentration less than the concentration of the calibrator.

2. Quantitative interpretation requires graphing the absorbances of the calibrators (X axis) versus the log of the calibrator concentration (Y axis) using software programs such as 4-parameters or alternatively logit-log. Samples with absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as < 0.04 ppb or >3.24 ppb, respectively.

3. To obtain the final concentration of the sample in honey, the concentration obtained in the assay needs to be multiplied by 50 to account for dilution factors in the extraction procedure.

   Alternatively, Abraxis can supply a spreadsheet template which can be used for data reduction. Please contact Abraxis for further details.

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