CALCULATE RESULTS

1. Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrator wells: Samples containing less color than a calibrator well 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 (Y axis) versus the log of the calibrator concentration (X axis) on semi-log graph paper. A straight line is drawn through the calibrator points and the sample absorbances are located on the line. The corresponding point on the X-axis is the concentration of the sample. Samples with absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as < 0.1 ppb or >2 ppb, respectively. Alternatively, Beacon can supply a spreadsheet template, which can be used for data reduction. Please contact Beacon for further details.

TECHNICAL ASSISTANCE

For questions regarding this kit or for additional information about Beacon products, call (207) 571-4302.

SAFETY

To receive complete safety information on this product, contact Beacon Analytical Systems, Inc. and request Material Safety Data Sheets. Stop Solution is 1N hydrochloric acid. Handle with care.

General Limited Warranty

Beacon Analytical Systems, Inc. (“Beacon”) warrants the products manufactured by it against defects in materials and workmanship when used in accordance with the applicable instructions for a period not to extend beyond a product’s printed expiration date. BEACON MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The warranty provided herein and the data, specifications and descriptions of Beacon products appearing in published catalogues and product literature may not be altered except by express written agreement signed by an officer of Beacon. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and, if given, should not be relied upon.

In the event of a breach of the foregoing warranty, Beacon’s sole obligation shall be to repair or replace, at its option, any product or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies Beacon promptly of any such defect. The exclusive remedy provided herein shall not be deemed to have failed of its essential purpose so long as Beacon is willing and able to repair or replace any nonconforming Beacon product or part. Beacon shall not be liable for consequential, incidental, special or any other indirect damages resulting from economic loss or property damage sustained by a customer from the use of its products. However, in some states the purchaser may have rights under state law in addition to those provided by this warranty.

BEACON ANALYTICAL SYSTEMS, INC.
82 Industrial Park Road
Saco, ME 04072
Tel. (207) 571-4302
Fax (207) 602-6502
www.beaconkits.com

Beacon Analytical Systems Inc.

Tetracycline Plate Kit
Cat. # 20-0143

Instructional Booklet

READ COMPLETELY BEFORE USE.

INTENDED USE

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

USE PRINCIPLES

The Beacon 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.

MATERIALS PROVIDED IN THE BEACON TETRACYCLINE PLATE KIT

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.

1 Plate containing 12 test strips of 8 wells each vacuum-packed in aluminized pouch with indicating desiccant.
1 vial containing “dry” calibrator stock.
1 vial of Calibrator diluent.
1 Tetracycline GAR HRP Enzyme Conjugate.
1 vial of Tetracycline antibody.
1 vial of Substrate.
1 vial of Stop Solution. (Caution! 1N HCl. Handle with care).
1 vial of 10X PBST wash solution.
1 Instructional Booklet.

PERFORMANCE CHARACTERISTICS

SPECIFICITY

The Beacon TETRACYCLINE Plate Kit cannot 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.

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: 15 ppb

MATERIALS REQUIRED BUT NOT PROVIDED

- Graduated cylinder, 100 ml or larger.
- Glassware for sample extraction and extract collection.
- Ultrasonic bath
- Pipet with disposable tips capable of dispensing 50 µL.
- Multi-channel pipet; 8 channel capable of dispensing 50 and 100 µL.
- Paper towels or equivalent absorbent material.
- Microwell plate or strip reader with 450nm filter.
- Timer
- Vortex mixer
- Wash bottle
- Laboratory quality distilled or deionized water.
- 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]

PRECAUTIONS

1. Each reagent is optimized for use in the Beacon Tetracycline Plate kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon 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 an antibiotic 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.

PREPARATION OF REAGENTS

Wash Solution: Add the contents of the 10 PBST wash solution bottle to a wash bottle and add 450mL of laboratory quality or deionized water. Swirl to mix.

Calibrator Preparation: Calibrators are made into calibrator diluent. **Prepare fresh daily.**

1. **50 ppm calibrator medium**: Add 1 mL of calibrator diluent to “dry” calibrator vial. Vortex to reconstitute and allow to sit.
2. **1.0 ppm calibrator medium**: Add 40 uL of 50 ppm to 2mL calibrator diluent.
3. **40.5 ppb calibrator**: Add 0.405mL of 1.0 ppm to 10mL of calibrator diluent.
4. **4.5 ppb calibrator**: Add 0.4mL of 40.5 ppb to 3.2mL of calibrator diluent.
5. **0.5 ppb calibrator**: Add 0.4mL of 4.5 ppb to 3.2mL of calibrator diluent
6. **0 ppb calibrator:** Calibrator diluent.

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.

ASSAY 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. Fill a wash bottle with lab grade water.
2. Place the appropriate number of test wells into a microwell holder. Be sure to re-seal unused wells in the zip-lock bag with desiccant.
4. Dilute all sample extracts/calibrators 1:10 in 10% MeOH/PBS.
3. Add 1**00 ul of Calibrators or Sample extract** to each well. 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.**
6. Dump the contents of the wells into an appropriate waste container. Fill the wells to overflowing with diluted wash solution and dump. Repeat 3X for a total of four washes.
7. Following the last wash tap the inverted wells onto absorbent paper to remove the last of the wash solution.
8. Using a pipette with disposable tips, add **200-µL enzyme conjugate** to the appropriate test wells.
9. Shake the plate gently for 30 seconds and incubate the test wells for 30 minutes.
10. Dump the contents of the wells into an appropriate waste container. Fill the wells to overflowing with diluted wash solution and dump. Repeat 3X for a total of four washes.
11. Following the last wash tap the inverted wells onto absorbent paper to remove the last of the wash solution.
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.