**Intended Use**

The Beacon Tetracycline Plate Kit is an immunoassay for the detection of Tetracycline in honey samples. This product is intended for research use only.

**Principles**

Calibrators and Sample Extract(s) are pipetted into the test wells followed by Rabbit α-Tetracycline Antibody. During an incubation, Rabbit α-Tetracycline Antibody binds to Tetracycline in the calibrator/sample which in turn is immobilized on the test wells surface. Following the incubation, the wells are washed to remove any non-specific binding. After washing, Goat α-Rabbit (GAR) HRP Enzyme Conjugate is added to each well. During an incubation, the GAR HRP Enzyme Conjugate binds any Rabbit α-Tetracycline Antibody present. After the incubation, the wells are washed to remove any non-specific binding. After washing, a colorless substrate is added to the wells and any bound enzyme conjugate will convert the substrate to a blue color. Following an incubation, the reaction is stopped with the addition of Stop Solution and the amount of color in each well is measured. The color of the unknown sample is compared to the color of the calibrators and the Tetracycline concentration of the sample is derived.

**Reagents and Materials Provided**

1 Plate containing 12 test strips of 8 wells each that are vacuum sealed in an aluminized pouch with a desiccant.
3 Vial of Lyophilized Calibrator Stock (50 µg/vial).
1 Bottle of GAR HRP Enzyme Conjugate.
1 Bottle of Tetracycline Antibody.
1 Bottle of Calibrator/Sample Diluent.
1 Bottle of 10X Wash Concentrate (dilute prior to use).
1 Bottle of Substrate.
1 Bottle of Stop Solution.

**Reagents and Materials Required but Not Provided**

- Pipette(s) with disposable tips capable of dispensing the required volume(s).
- Multichannel pipette(s) (8 channels) with disposable tips capable of dispensing the required volume(s) (recommended if running more than two strips at once).
- Laboratory quality distilled or deionized water.
- Reagents and materials for sample preparation.
- Materials for 1X wash solution preparation.
- Personal protective equipment.
- Paper towels or equivalent absorbent material.
- Timer.
- Microtiter plate or strip reader capable of reading at 450 nm.

**Kit Handling Notes and Precautions**

- Read the product brochure in its entirety prior to use.
- The kit, in its original packaging, can be used until the end of the month indicated on the box label.
- Do not use reagents after expiration date.
- Store all kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Reagents should be brought to room temperature, 20°C to 28°C (62°C to 82°F), prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
- Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).
- Running Calibrators and Samples in duplicate will improve assay precision and accuracy.
- Precise transfer of samples and reagents by using a calibrated pipette that is capable of dispensing the required volume is critical to obtain proper assay results.
- If running more than two strips at once, the use of a multi-channel pipette is recommended when adding the Antibody, Substrate and Stop Solution.
- All procedural steps should be completed without interruption. Ensure all reagents, materials and equipment are ready at the appropriate time.
- 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.
- Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
- Damage to or obstruction of the optical surface may cause unsatisfactory results.

**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 percent cross reactivity versus Tetracycline.

| Compound | % Cross-Reactivity |
|---|---|
| Tetracycline | 100 |
| Rolitetracycline | 97 |
| Chlorotetracycline-HCl | 90 |
| Demeclocycline-HCl | 13 |
| Oxytetracycline | 1.4 |
| Minocycline | 0.7 |
| Doxycycline Hyclate | 0.5 |

**Limit of Detection (LOD)**

| Matrix | LOD (ppb) |
|---|---|
| Honey | 15 |

**Sample Extraction Buffer Preparation (20 mM PBS)**

1. Measure 1 L of laboratory quality distilled or deionized water and add to a clean container with a tight fitting lid.
2. Weigh 2.76 g of $NaH_2PO_4 \cdot H_2O$ (F.W. 137.99) and add to the container.
3. Weigh 8.5 g of NaCl and add to the container.
4. Gently stir to mix.
5. Measure the pH of the solution and adjust to achieve a pH of 5, as necessary.

**Sample Preparation**

Honey: (Dilution Factor: 50)

1. Weigh 1 gram of the sample in a 60 – 80 mL glass container with a screw cap.
2. Measure 49 mL of Sample Extraction Buffer and add to the container.
3. Place the container in an ultrasonic water bath for 5 minutes.
4. Mix vigorously for 2 minutes.
5. Dilute an aliquot of the extract 1:10 in Calibrator/Sample Diluent. Mix thoroughly and use in the assay.

**Calibrator Preparation**

Note: Calibrators must be prepared fresh daily. Tightly cap vials to prevent evaporation. Thoroughly mix calibrators in-between dilution steps and prior to use.

1. Prepare a 50 ppm calibrator stock by reconstituting the lyophilized calibrator stock (50 µg/vial) in 1 mL of Calibrator/Sample Diluent. Vortex vigorously for 30 seconds. Allow to sit for 10 minutes before proceeding. Vortex vigorously for 30 seconds again.
2. Prepare a 1 ppm intermediate calibrator stock by diluting an aliquot of the 50 ppm calibrator stock 1:50 in Calibrator/Sample Diluent. Vortex vigorously for 30 seconds before proceeding.
3. Prepare the calibrators as follows:

| Calibrator Concentration | Calibrator/Sample Diluent Volume | Stock Volume | Stock Description |
|---|---|---|---|
| 40.5 ppb | 10 mL | 0.405 mL | 1 ppm Intermediate Stock |
| 4.5 ppb | 3.2 mL | 0.4 mL | 40.5 ppb |
| 0.5 ppb | 3.2 mL | 0.4 mL | 4.5 ppb |
| 0 ppb | 1.0 mL | N/A | N/A |

4. Dilute the calibrators 1:10 in Calibrator/Sample Diluent. Mix thoroughly and use in the assay.

**1X Wash Solution Preparation**

1. Measure 450 mL of laboratory quality distilled or deionized water and transfer to a clean container with a tight-fitting lid.
2. Transfer the contents of the 10X Wash Concentrate bottle to the container.
3. Gently swirl to mix.
4. Transfer the 1X Wash Solution to a wash bottle to use in the assay.

**Assay Procedure**

1. Allow kit components and the sample extract(s) to reach room temperature prior to running the test.
2. Place the appropriate number of test wells into a holder. Be sure to re-seal unused test wells in the zip-lock bag with the desiccant to limit exposure to moisture.
3. Dispense **100 µL of Calibrators and Sample Extract(s)** into the appropriate well. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
4. Dispense **100 µL of Antibody** into each well.
5. Gently shake the wells for 30 seconds using a back-and-forth motion and incubate for **30 minutes** at room temperature.
6. Decant the contents of the wells into an appropriate waste container. Fill the wells to overflowing with 1X Wash Solution and then decant. Repeat this wash step four times for a total of five washes. Following the last wash, tap the inverted wells onto absorbent paper to remove excess wash solution.
7. Dispense **200 µL of Enzyme Conjugate** into each well.
8. Incubate the test wells for **30 minutes** at room temperature.
9. Decant the contents of the wells into an appropriate waste container. Fill the wells to overflowing with 1X Wash Solution and then decant. Repeat this wash step four times for a total of five washes. Following the last wash, tap the inverted wells onto absorbent paper to remove excess wash solution.
10. Dispense **100 µL of Substrate** into each well.
11. Incubate for **30 minutes** at room temperature.
12. Dispense **100 µL of Stop Solution** into each well in the same order of addition as the Substrate.
13. Gently shake the wells for 30 seconds using a back-and-forth motion.
14. Carefully wipe the optical surface with a soft, lint-free wipe. Measure and record the absorbance (Optical Density; OD) of each well at 450 nm using a plate or strip reader within 10 minutes of stopping the assay. If the reader has dual wavelength capability, read at 450 nm minus 605 or 650 nm.

**Result Interpretation**

Semi-Quantitative Interpretation: Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrators:

- Samples with a lower absorbance (less color) than a calibrator have a concentration of Tetracycline greater than the concentration of the calibrator.
- Samples with a higher absorbance (more color) than a calibrator have a concentration less than the concentration of the calibrator.

Quantitative Interpretation: It is preferred for quantitative results to be determined using commercially available software for ELISA evaluation using a 4-parameter curve fit. Alternatively, a semi-log curve fit can be used if 4-parameter software is not available. A spreadsheet that will perform the curve fit and sample concentration calculations is available upon request. Please contact Beacon for further details.

- The concentration of Tetracycline in a sample is determined by comparing the average sample absorbance to the standard curve. This value must then be multiplied by the dilution factor used.
- Samples with absorbances lower than the highest calibrator contain a concentration of Tetracycline too high for quantification. Further dilute the sample extract in Calibrator/Sample Diluent to fit into the standard curve and retest along with the calibrators. Results must then be multiplied by the dilution factor used.
- Samples with Tetracycline absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as < 0.05 ppb or > 4.05 ppb, respectively.

**Technical Assistance**

For questions regarding this kit or for additional information about Beacon products, contact us.

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**Safety**

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

**General Limited Warranty**