Abraxis Tylosin Plate Kit

PN 52256B

Instructional Booklet
Read Completely Before Use.

INTENDED USE

The Tylosin Plate Kit is a competitive ELISA for the quantitative analysis of Tylosin in water samples and honey products.

ASSAY PRINCIPLES

The Tylosin plate kit is a competitive enzyme-labeled immunoassay. Stabilized water samples or extracted honey samples and Tylosin calibration standards are pipetted into the test wells followed by Tylosin-HRP conjugate. Anti-Tylosin antibody is then pipetted into the test wells to initiate the reaction. During the 60 minute incubation period, Tylosin, when present in a sample, and Tylosin-HRP conjugate compete for the binding sites of the anti-Tylosin antibody in solution. The Tylosin antibodies are bound by a secondary antibody immobilized on the microtiter plate. Following this incubation, the contents of the wells are decanted and the wells are washed to remove any unbound reagents. A substrate is added to the wells and any bound enzyme conjugate causes the conversion to a blue color. Following a 20-30 minute incubation, the reaction is stopped and the amount of color in each well is read. The color of the unknown samples is compared to the color of the calibration standards and the Tylosin concentration of the samples is determined by interpolation.

SPECIFICITY

The Tylosin Plate Kit cannot differentiate between the various Tylosins, but detects their presence to differing degrees. The following table shows the % cross reactivity of Tilmicosin versus Tylosin.

Compound	**% CR**
Tylosin	**100%**
Tilmicosin	**125%**

ASSAY DETECTION LIMIT

0.05 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.

- Microtiter plate containing 12 test strips of 8 wells sealed in a re-sealable pouch with desiccant.
- 1 bottle containing 25 mL of Stabilizer/Sample Diluent (10X concentrated). This solution, when used as a water sample stabilizer, must be added at a 1:10 ratio to the water sample (i.e. 100 µL of 10X stabilizer to 900 µL of water sample). When used to extract honey samples or dilute samples above the range of calibration, it must be diluted 1:10 in deionized (or distilled) water before use (i.e. 100 µL of 10X Stabilizer/Sample Diluent to 900 µL of deionized water).
- 7 vials containing 1.0 mL of ready to use Tylosin calibration standards corresponding to 0; 0.1; 0.25; 0.50; 1; 2.5; and 5 ppb.
- 1 bottle containing 6 mL of Tylosin-HRP Enzyme Conjugate.

- 1 bottle containing 6 mL of anti-Tylosin Antibody.
- 1 bottle containing 100 mL of 5X Wash Buffer Concentrate. The wash solution provided is a 5X concentrated solution and needs to be diluted 1:5 with deionized water. If using the entire bottle, add to 400 mL of deionized water. This diluted solution is used to wash the wells of the microtiter plate.
- 1 bottle containing 12 mL of Substrate (Color Solution).
- 1 bottle containing 12 mL of Stop Solution. (Caution! 1N HCl. Handle with care.)
- Instructions

PRECAUTIONS

1. Each reagent is optimized for use in the Tylosin Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Abraxis Tylosin 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. Tylosin is an antibiotic and should be treated with care.
6. The Stop Solution is 1N hydrochloric acid. Avoid contact with skin and mucous membranes. If contact should occur, immediately flush with copious amounts of water. Immediately clean up any spills and wash area with copious amounts of water.
7. The wash solution provided is a 5X concentrated solution and needs to be diluted 1:5 with deionized water. If using the entire bottle (100 mL) add to 400 mL of deionized water. This diluted solution is used to wash the wells of the microtiter plate.
8. It has been found that Tylosin in water samples will adhere to vessel walls. Therefore, collect samples in glass bottles and preserve immediately by adding 100 µL of the concentrated (10X) Stabilizer/Sample Diluent (as provided) per 900 µL of water sample.
9. The Stabilizer/Sample Diluent is provided as a 10X concentrated solution. When used to extract honey samples or dilute samples above the range of calibration, it must be diluted 1:10 in deionized (or distilled) water before use (i.e. 100 µL of 10X Stabilizer/Sample Diluent to 900 µL of deionized water).

MATERIALS REQUIRED BUT NOT PROVIDED

1. Laboratory quality distilled or deionized water.
2. Graduated cylinder, 100 mL or larger.
3. Glassware for sample collection.
4. 4 mL glass vials with Teflon-lined caps for honey sample extraction and extract dilution.
5. Pipet with disposable tips capable of dispensing 50 µL.
6. Multi-channel pipet or electronic repeating pipette with disposable tips (capable of dispensing 50-250 µL)
7. Reagent basins
8. Paper towels or equivalent absorbent material.
9. Microtiter plate or strip reader with 450nm filter.
10. Timer

11. Vortex mixer
12. Container or wash bottle, 500 mL or larger, for dilution of wash solution

WATER SAMPLE PREPARATION

Collect water samples in glass bottles and preserve immediately by adding 100 µL of the concentrated (10X) Stabilizer/Sample Diluent (as provided) per 900 µL of water sample.

HONEY SAMPLE EXTRACTION (1:25 dilution)

1. Weigh 1 gram of honey into a 4 mL glass vial with Teflon-lined screw cap.
2. Add 1 mL of 1X Stabilizer/Sample Diluent.
3. Put the sample vial in an ultrasonic water bath for 5 minutes.
4. Mix vigorously for 2 minutes.
5. Dilute sample 1:12.5 as follows: 50 µL of sample (step 4) and 575 µL of 1X Stabilizer/Sample Diluent.
6. Invert the sample vial several times to mix; immediately transfer 50 µL into duplicate wells for the assay.

TEST PROCEDURE (Note: Running calibration standards and samples in duplicate is recommended as it will improve assay precision and accuracy.)

1. Allow reagents and sample extracts to reach room temperature prior to running the test.
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 desiccant.
3. Using a pipet with disposable tips, add **50 µL of calibration standards and samples** to the appropriate test wells. Be sure to use a clean pipet tip for each.
4. Using a multi-channel or repeating pipette, dispense **50 µL of Enzyme Conjugate** into each test well.
5. Using a multi-channel or repeating pipette, dispense **50 µL of Antibody Solution** into each test well.
6. Mix the contents of the wells by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate at room temperature for **60 minutes**.
7. Decant the contents of the wells into an appropriate waste container. Fill the wells with at least 250 µL of 1X wash buffer (or if using a wash bottle, to overflowing) and decant. Repeat three times for a total of four washes.
8. Following the last wash, tap the inverted wells onto paper towels or other absorbent material to remove the last of the wash solution.
9. Using a multi-channel or repeating pipette, dispense **100 µL of Substrate (Color Solution)** into each well.
10. Mix the contents of the wells by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the wells for **20-30 minutes**.
11. Using a multi-channel or repeating pipette, dispense **100 µL of Stop Solution** into each test well.
12. 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 absorbances of the calibration standard wells: A sample well containing less color than a calibration standard well will have a Tylosin concentration greater than the concentration of the calibration standard. A sample well containing more color than a calibration standard well will have a Tylosin concentration less than the concentration of the calibration standard.

2. Quantitative interpretation requires graphing the absorbances of the calibration standard (X axis) versus the log of the calibration standard concentration (Y axis) on semi-log graph paper. A straight line is drawn through the calibration standard points and the sample absorbances are located on the line. The corresponding point on the Y axis is the concentration of the sample. Samples with absorbances greater than the lowest calibration standard should be assumed to be below the detection limit of the assay. Samples with absorbances less than the highest calibration standard must be diluted to obtain accurate quantitative results.
3. Results obtained with freshwater samples which have been preserved with 10X Stabilizer/Sample Diluent as described above must be multiplied by a factor of 1.1 to account for the initial dilution of samples with the 10X Sample Stabilizer. Samples having a higher absorbance than Standard 1 (0.1 ppb) should be reported as <0.11 ppb. Samples having a lower absorbance than Standard 6 (5 ppb) should be reported as >5.5 ppb or diluted with 1X Stabilizer/Sample Diluent and re-analyzed to obtain accurate quantitative results.
4. Results obtained with extracted honey samples must be multiplied by a dilution factor of 25 to account for the overall dilution of the extraction procedure. Samples having a higher absorbance than Standard 1 (0.1 ppb) should be reported as <2.5 ppb. Samples having a lower absorbance than Standard 6 (5 ppb) should be reported as >125 ppb or diluted with 1X Stabilizer/Sample Diluent and re-analyzed to obtain accurate quantitative results.

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

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