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 honey products.

ASSAY PRINCIPLES

The Tylosin plate kit is a competitive enzyme-labeled immunoassay. Tylosin is extracted from a sample by blending or shaking with extraction solution. The Tylosin sample extract and calibrators are pipetted into the test wells followed by Tylosin antibody into the test wells to initiate the reaction. During the 30 minute incubation period, Tylosin from the sample and Tylosin HRP conjugate compete for binding to Tylosin antibody. Following this 30 minute incubation, the contents of the well are removed and the wells are washed to remove any unbound Tylosin, Tylosin HRP conjugate and free Tylosin antibody. After 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 the amount of color in each well is read. The color of the unknown samples is compared to the color of the calibrators and the Tylosin concentration of the samples is derived.

SPECIFICITY:

The Tylosin Plate Kit can not differentiate between the various Tylosins, but detects their presence to differing degrees. The following table shows the % cross reactivity of Tilmicosin versus Tylosin. All concentrations are in parts per billion (ppb).

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

DETECTION LIMIT:

Honey: 1.25 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 Tylosin calibrators corresponding to 0, 0.05, 0.1, 0.5, 1, 5 µg/L (ppb) of Tylosin.
- 1 vial containing 7 mL Tylosin HRP Enzyme Conjugate.
- 1 vial containing 7 mL of anti-Tylosin antibody.
- 1 vial containing 14 mL of Substrate.
- 1 vial containing 14 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. 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. Methanol
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 7.4. [0.62 g NaH_2PO_4-$2H_2O$+ 5.73 g Na_2HPO_4-$12H_2O$ + 9 g NaCl, /liter, pH 7.4]

HONEY SAMPLE EXTRACTION (1:25 dilution)

1. Weigh 1 gram of honey in a screw cap glass bottle (50ml size)
2. Add 24 ml of 20 mM PBS (1ml sample + 24 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 transfering 50 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 reach room temperature prior to running the test. Fill a wash bottle with 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 **50 µL enzyme conjugate** to the appropriate test wells. Be sure to use a clean pipet tip for each. Add **50 ul of Calibrators or Sample extract** to each well

4. Dispense **50 µL of Antibody Solution** into each test well.

5. Shake the plate gently for 30 seconds and incubate the test wells for **60 minutes**.

7. Dump the contents of the wells into an appropriate waste container. Fill the wells to overflowing with tap water 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. Dispense **100 µL of Substrate** into each well.

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

11. 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 absorbance of the calibrator wells: Sample containing less color than a calibrator well having a concentration of Tylosin 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) 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 Y 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 < 0.05 ppb or >5 ppb, respectively.

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

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