Abraxis Avermectins Plate Kit
For Analysis of Ivermectin, Abamectin, and Dormectin

Cat. # 5142B

Instructional Booklet
Read Completely Before Use.

INTENDED USE

The Abraxis Avermectins Plate Kit is a competitive ELISA for the quantitative analysis of Avermectins in honey products.

ASSAY PRINCIPLES

The Abraxis Avermectins plate kit is a competitive enzyme-labeled immunoassay. Avermectins is extracted from a sample by blending or shaking with extraction solution. The sample extract and calibrators are pipetted into the test wells followed by Avermectins-HRP conjugate solution. An Avermectins antibody solution is then added into the test wells to initiate the reaction. During the 30 minute incubation period, Avermectins from the sample and Avermectins HRP conjugate compete for binding to Avermectins antibody. The Avermectin antibodies are bound by a second antibody immobilized on the microtiter plate wells. Following a 30 minute incubation, the contents of the wells are removed and the wells are washed to remove any unbound Avermectins, and Avermectins HRP conjugate. 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 Avermectins concentration of the samples is derived by interpolation using a standard curve constructed with each run.

SPECIFICITY:

The Abraxis Avermectins Plate Kit can not differentiate between the various Avermectinss, but detects their presence to differing degrees. The following table shows the % cross reactivity of Ivermectin and Dormectin versus Abamectin. All concentrations are in parts per billion (ppb).

Compound	% CR
Abamectin	**100%**
Ivermectin	**61%**
Dormectin	**32%**

DETECTION LIMIT:
Honey: 20 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.
- Abamectin calibrators (6) corresponding to 0, 0.185, 0.560, 1.7, 5, 15 µg/L (ppb) of Abamectin.
- 1 vial containing 7 mL Avermectins HRP Enzyme Conjugate.
- 1 vial containing 7 mL of Polyclonal anti-Avermectins antibody.
- 1 bottle containing 100 mL of Wash Solution (5X). It must be diluted with 400 mL of deionized water before use.
- 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 Abraxis Avermectins Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Abraxis Avermectins 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. Avermectins are 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. Methanol
5. Pipet with disposable tips (10-200 µL).
6. Multi-channel pipet; 8 channel or stepper pipette capable of dispensing (10-250 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. J. T. Baker C18 column (7020-01).

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.
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.
4. Add **50 ul of Calibrators or Sample extract** to the appropriate well using a pipette with disposable tips Be sure to use a clean tip for each sample.
4. Dispense **50 µL of Antibody Solution** into each test well using a multi-channel or stepping pipette.
5. Cover the wells with parafilm or tape and mix the plate gently in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the test wells for **60 minutes**.
7. Deacnt the contents of the wells into an appropriate waste container. Wash the wells with 250 uL of 1X Wash Solution and decant. 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 have a concentration of Avermectins 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 < 0.185 ppb or >15 ppb, respectively.

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

GENERAL LIMITED WARRANTY

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