4. PERFORMANCE DATA

Test Sensitivity
Honey: 20 ppb

Test reproducibility:
The standard curve R^2 must be ≥ 0.98. The absorbance Coefficient of variation (CVs) for standards should be ≤10% and for the samples should be ≤15%. The Standard 0 absorbance value should be between 0.8 - 3.000.

Cross Reactivities:
The Avermectins Plate Kit cannot differentiate between the various Avermectins but detects their presence to differing degrees. The following table shows the % cross reactivity of Ivermectin, Eprinomectin, and Dormectin versus Abamectin.

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

General Limited Warranty:
Gold Standard Diagnostics warrants the products manufactured by the Company against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. **Gold Standard Diagnostics makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.**

For ordering or technical assistance contact:

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GOLD STANDARD DIAGNOSTICS

Avermectins ELISA Microtiter Plate

For Analysis of Ivermectin, Abamectin, Eprinomectin, and Dormectin
Product No. 5142B

1. INTENDED USE

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

2. ASSAY PRINCIPLES

The Avermectins plate kit is a competitive enzyme-labeled immunoassay. Avermectins are extracted from a sample by blending or shaking with extraction solution. The sample extracts and calibrators are pipetted into the test wells followed by an 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 the Avermectins-HRP conjugate compete for binding to the Avermectins antibodies. The Avermectins antibodies are then bound by a second antibody immobilized on the microtiter plate. Following a 30-minute incubation, the contents of the well are removed and the wells are washed to remove any unbound Avermectins, Avermectins-HRP conjugate, and free Avermectins antibody. A clear substrate is then added to the wells and the 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 concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

3. WORKING INSTRUCTIONS

A. 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.

1. Microtiter plate containing 12 test strips of 8 wells in an aluminized pouch with desiccant
2. Avermectins calibrators (6) containing 0, 0.185, 0.560, 1.7, 5.0, and 15.0 µg/L (ppb) of Abamectin
3. Avermectins-HRP Enzyme Conjugate Solution, 7 mL
4. Polyclonal Anti-Avermectins Antibody Solution, 7 mL
5. ABRAXIS® Wash Solution (5X) Concentrate, must be diluted 1:5 with deionized or distilled water before use
6. Color (Substrate) Solution, 14 mL
7. Stop Solution, 14 mL. (Caution! 1N HCl. Handle with care)
8. Instructions

B. 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. Pipette with disposable tips (10-200 µL)
6. Multi-channel pipette or stepper pipette (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. J. T. Baker C18 column (7020-01)

C. Precautions

1. Each reagent is optimized for use in the Avermectins Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other 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. Reagents may be used until the expiration date on the box.
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. If contact should occur, immediately flush with copious amounts of water. Immediately clean up any spills and wash area with copious amounts of water

D. Test 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. Dilute 5X concentrated Wash Solution 1:5 with distilled or deionized 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 desiccant.
3. **Dispense 50 µL of enzyme conjugate** to each of the test wells using a multi-channel or stepping pipette.
4. **Add 50 µL of the appropriate Calibrator or Sample Extract** to the appropriate well using a pipette with disposable tips. Be sure to use a clean tip for each.
5. **Dispense 50 µL of Antibody Solution** into each test well using a multi-channel or stepping pipette.
6. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. **Incubate the test wells for 30 minutes**.
7. Decant the contents of the wells into an appropriate waste container. **Wash the wells with 250 µL of 1X Wash Solution 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 remaining water.
9. **Dispense 100 µL of Color (Substrate) Solution** into each well using a multi-channel or stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents.
10. **Incubate the wells for 30 minutes**.
11. **Dispense 100 µL of Stop Solution** into each well using a multi-channel or stepping pipette.
12. Read and record the absorbance of the wells at 450nm using a strip or plate reader.

E. Results interpretation

1. Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbances of the calibrator wells. Sample wells containing less color than a calibrator well will have an Avermectins concentration greater than the concentration of the calibrator. Sample wells containing more color than a calibrator well have an Avermectins concentration that is 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, Gold Standard Diagnostics can supply a spreadsheet template which can be used for data reduction. Please contact Gold Standard Diagnostics for further details.