Beacon Analytical Systems Inc.

Ivermectin HS Plate Kit

Cat.# 20-0272

(FOR ANALYSIS OF PLASMA SAMPLES)

Instructional Booklet

READ COMPLETELY BEFORE USE.

INTENDED USE

The Beacon Ivermectin HS Plate Kit is a competitive ELISA for the quantitative analysis of Ivermectin and related compounds in plasma samples.

USE PRINCIPLES

The Beacon Ivermectin HS plate kit is a competitive enzyme-labeled immunoassay. Ivermectin HRP conjugate is pipetted into the test wells followed by sample extract and calibrators. An Ivermectin antibody solution is then added into the test wells to initiate the reaction. During a 30 minute incubation period, Ivermectin from the sample and Ivermectin HRP conjugate compete for binding to the Ivermectin antibody. Following this incubation, the wells are washed to remove any unbound Ivermectin and Ivermectin HRP conjugate. After washing, a colorless substrate is added to the wells and any bound enzyme conjugate will convert the substrate to a blue color. Following another 30 minute 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 Ivermectin concentration of the sample is derived.

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 to 8ºC.

- 1 plate containing 12 test strips of 8 wells each vacuum-packed in aluminized pouch with indicating dessicant
- 1 vial containing 6 mL of Extraction Buffer
- 1 bottle containing 40 mL of Dilution Buffer for **Plate** assay (Caution! Contains organic solvent)
- 1 vial of Negative control (0 ppb Ivermectin)
- 3 vials each containing 4 mL of Ivermectin calibrators with a concentration of 0.1, 0.6, and 3.6 µg/L (ppb) of Ivermectin
- 1 vial containing 8 mL of Ivermectin-HRP Enzyme Conjugate
- 1 vial containing 8 mL of Anti-Ivermectin Antibody

- 1 vial containing 14 mL of Substrate
- 1 vial containing 14 mL of Stop Solution (Caution! 1N HCl. Handle with care.)
- 1 packet of Wash Solution salts
- 1 instructional booklet

MATERIALS REQUIRED BUT NOT PROVIDED

- Laboratory quality distilled or deionized water
- Acetonitrile (ACS grade)
- Sample extraction or dilution tube (culture tube 12 X 75 mm or equivalent)
- Becton, Dickinson and Company (BD) Vacutainer K2 blood collection EDTA tube (BD cat.# 367899) or equivalent
- Pipette with tips capable of dispensing 50 to 200 µL
- Pipette with tips capable of dispensing 500 to 1000 µL
- Multi-channel pipette; 8 channels capable of dispensing 50 µL
- Paper towels or equivalent absorbent material
- Micro well plate or strip reader with 450 nm filter
- Timer
- Vortex mixer
- Wash bottle

SPECIFICITY

Ivermectin belongs to the Avermectin drug family. A number of Avermectin drugs can be detected by this assay. The % cross reactivity of several Avermectin drugs relative to Ivermectin is shown in the table below.

Compound	% CR
Ivermectin	**100%**
Abamectin	**160%**
Avermectin B1a	**167%**
Avermectin B1b	**109%**
Doramectin	**37%**
Eprinomectin	**141%**

PRECAUTIONS

- Store all kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Each reagent is optimized for use in the Beacon Ivermectin HS Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Ivermectin HS Plate Kits with different lot numbers. Do not use the dilution buffer in Beacon's Ivermectin Tube Kit (Cat.# 20-0285) for this assay.
- Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
- Do not use reagents after expiration date.
- Reagents should be brought to room temperature, 20 to 28ºC (62 to 82ºF) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
- Ivermectin is a toxic antiparasitic drug and should be treated with care.
- The Stop Solution is 1N hydrochloric acid, which is corrosive and an irritant. 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.
- Transfer of samples and reagents by pipette requires constant monitoring of technique. Pipetting errors are the major source of error in immunoassay methodology.
- Keep Calibrators and Dilution Buffer bottles tightly capped when not in use to prevent evaporation of organic solvent in the solutions.
- If running more than two strips at once, the use of a multichannel pipette is required.

SAMPLE PREPARATION (EDTA whole blood)

1. Allow Extraction Buffer and Dilution Buffer to reach room temperature prior to use (Approximately 1 – 2 hours). Shake the Extraction Buffer until the crystals in the solution dissolve completely.
2. **Collect** animal blood using a K2 EDTA blood collection tube.
3. **Separate** plasma from red blood cells by centrifugation at 1000 x g for 10 minutes.
4. **Transfer 0.5 mL** of the plasma sample into a glass tube.
5. **Add 0.1 mL** of Extraction Buffer and vortex for 10 seconds.
6. **Add 0.9 mL** of 100% acetonitrile.
7. **Vortex for 1 minute to mix.**
8. **Let sample stand** until clear top layer appears. This will take approximately 30 seconds.
9. **Dilute** the clear top layer of sample (1:5) with Dilution Buffer (0.2 mL of supernatant + 0.8 mL of Dilution Buffer).
10. **Vortex for 30 seconds to mix** (at high speed).
11. Prepare sample immediately before analysis to eliminate evaporation that may occur upon standing at room temperature.
12. If the absorbance is lower than the highest calibrator (3.6 µg/L), the concentration of Ivermectin is too high, then dilute extract in 60% methanol/water and rerun.

ASSAY 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 (Approximately 1 – 2 hours).
2. Prepare the wash solution by transferring the contents of the Wash Solution packet to 1 liter of laboratory grade water. Swirl to mix. Transfer the diluted wash solution to a wash bottle.
3. Place the appropriate number of test wells into a micro well holder. Be sure to re-seal unused wells in the zip-lock bag with desiccant.
4. Dispense **50 µL of HRP Enzyme Conjugate** into each well.
5. Add **50 µL of Calibrators or Sample Extract** to the appropriate wells. Be sure to use a clean pipette tip for each. (Keep calibrator vials capped when not in use.)
6. Dispense **50 µL of Antibody Solution** into each well.
7. Shake the plate gently for 30 seconds using a back and forth motion and then incubate the wells for **30 minutes** at room temperature.
8. Decant the contents of the wells into an appropriate waste container. Fill the wells to overflowing with working wash solution and then decant. Repeat 3X for a total of four washes.
9. Following the last wash, tap the inverted wells onto absorbent paper to remove the last of the wash solution.
10. Dispense **100 µL of Substrate** into each well.
11. Incubate the wells for **30 minutes** at room temperature.
12. Dispense **100 µL of Stop Solution** into each well.
13. Measure and record the absorbance (Optical Density; OD) of the wells at 450 nm using a strip or plate reader.
14. To obtain the plasma concentrations of Ivermectin multiply the results by a factor of **15**.

CALCULATE RESULTS

1. Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrator wells: Sample s containing less color than a calibrator will have a concentration of Abamectin greater than the concentration of the calibrator. Samples containing more color than a calibrator will have a concentration less than the concentration of the calibrator.

2. It is preferred for quantitative results to be determined using commercially available software for ELISA evaluation such as a 4-Parameter curve fit. Alternatively, a semi-log curve fit can be used if 4-Parameter software is not available. Samples with absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as

< 1.5 ppb or > 54 ppb, respectively. Alternatively, Beacon can supply a spreadsheet template which can be used for data reduction. Please contact Beacon for further details.

SAMPLE CALCULATIONS

Well Contents	OD	Average OD ± SD*	%RSD	%Bo**	Plasma Conc.
Negative Control	1.850 1.909	1.879 ± 0.041	2.2	100	0 ppb
0.1 ppb Calibrator	1.640 1.592	1.616 ± 0.034	2.1	86	1.5 ppb
0.6 ppb Calibrator	1.036 1.032	1.034 ± 0.003	0.3	55	9.0 ppb
3.6 ppb Calibrator	0.459 0.440	0.449 ± 0.013	3.0	24	54 ppb

Actual values may vary; this data is for example purposes only.

* standard deviation

** %Bo equals average sample absorbance divided by average negative control absorbance times 100.

TECHNICAL ASSISTANCE

For questions regarding this kit or for additional information about Beacon products, call (207) 571-4302 or local representative.

SAFETY

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

General Limited Warranty

Beacon Analytical Systems, Inc. (“Beacon”) warrants the products manufactured by it against defects in materials and workmanship when used in accordance with the applicable instructions for a period not to extend beyond a product’s printed expiration date. BEACON MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The warranty provided herein and the data, specifications and descriptions of Beacon products appearing in published catalogues and product literature may not be altered except by express written agreement signed by an officer of Beacon. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and, if given, should not be relied upon.

In the event of a breach of the foregoing warranty, Beacon’s sole obligation shall be to repair or replace, at its option, any product or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies Beacon promptly of any such defect. The exclusive remedy provided herein shall not be deemed to have failed of its essential purpose so long as Beacon is willing and able to repair or replace any nonconforming Beacon product or part. Beacon shall not be liable for consequential, incidental, special or any other indirect damages resulting from economic loss or property damage sustained by a customer from the use of its products. However, in some states the purchaser may have rights under state law in addition to those provided by this warranty.

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