



## Ivermectin Plate Kit

Cat.# 20-0224

Product Insert

READ COMPLETELY BEFORE USE.

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### INTENDED USE

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

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### USE PRINCIPLES

The Beacon Ivermectin plate kit is a competitive enzyme-labeled immunoassay. Ivermectin HRP enzyme conjugate is pipetted into the test wells followed by calibrators or sample extract. 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. The color intensity is inversely proportional to the amount of Ivermectin present.

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### 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 8°C.

- **Plate** – (1) containing 12 test strips of 8 wells each vacuum-packed in aluminized pouch with indicating desiccant
- **Negative control (0 ppb Ivermectin)** – (1) vial containing 2 mL
- **Ivermectin Calibrators** – (5) vials each containing 2 mL with a concentration of 0.5, 2, 5, 8 and 32 µg/L (ppb) of Ivermectin
- **Ivermectin HRP Enzyme Conjugate** – (1) vial containing 8 mL
- **Ivermectin Antibody Solution** – (1) bottle containing 8 mL
- **Substrate** – (1) vial containing 14 mL
- **Stop Solution** – (1) vial containing 14 mL (Caution! Contains 1N HCl. Handle with care.)

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## MATERIALS REQUIRED BUT NOT PROVIDED

- Methanol (ACS grade)
- Laboratory quality distilled or deionized water
- Pipette with disposable tips capable of dispensing 50 µL
- Multi-channel pipette; 8 channel capable of dispensing 50 and 100 µL
- Paper towels or equivalent absorbent material
- Micro well plate or strip reader with 450 nm filter
- Timer
- Wash bottle
- Vortex mixer

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## 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	167%
Dormectin	38%
Eprinomectin	170%
Abamectin B1a	188%
Abamectin B1b	83%

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## 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 Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Ivermectin Plate Kits with different lot numbers.
- 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 28°C (62 to 82°F) 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.
- Precise transfer of samples and reagents by using an appropriate and calibrated pipette is critical to obtain proper assay results. Please pipette carefully.
- If running more than two strips at once, the use of a multichannel pipette is required.

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## SAMPLE PREPARATION (milk: dilution factor 2.5)

1. Add 1 mL of milk sample with 1.5 mL of methanol in a small test tube. Mix using a vortex mixer.
2. Transfer 1.0 mL of the treated sample into a microcentrifuge tube. Centrifuge the tube for 5 minutes at 2,000 x g or equivalent to pellet the precipitate.
3. The supernatant is ready to test. Transfer it to a clear glass vial for storage at 4°C to 8°C (39°F to 46°F) when not in use.

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## 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.
2. 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.
3. Dispense **50 µL of the HRP Enzyme Conjugate** into each well.
4. Using a pipette with disposable tips, add **50 µL of the Calibrators or sample extract** into the appropriate test wells. Be sure to use a clean pipette tip for each.
5. Dispense **50 µL of the Antibody Solution** into each well.
6. Shake the plate gently for 30 seconds using a back and forth motion. Then incubate the wells for **30 minutes** at room temperature.
7. Decant the contents of the wells into an appropriate waste container. Fill the wells to overflowing with laboratory grade water and then decant. Repeat 4X for a total of five washes.
8. Following the last wash, tap the inverted wells onto absorbent paper to remove the last of the water.
9. Dispense **100 µL of the Substrate** into each well. Shake the plate gently for 30 seconds using a back and forth motion.
10. Incubate the wells for **30 minutes** at room temperature.
11. Dispense **100 µL of the Stop Solution** into each well.
12. Measure and record the absorbance (Optical Density; OD) of the wells at 450 nm using a strip or plate reader.
13. To obtain the concentration of Ivermectin in the sample multiply the results by the dilution factor.
14. If the absorbance is lower than the highest calibrator (32 µg/L), the concentration of Ivermectin is too high, then dilute the sample extract in 60% methanol/water and retest.

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## CALCULATE RESULTS

1. Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrator wells. Samples containing less color than a calibrator will have a concentration of Ivermectin 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 that quantitative results from the ELISA are determined using commercially available software using a 4-Parameter curve fit. Alternatively, a semi-log curve fit can be used if 4-Parameter software is not available. Milk samples with absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as < 1.25 ppb or > 80 ppb, respectively. 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 $\pm$ SD*	%RSD	%Bo**	Milk Conc. (ppb)
Negative Control	1.9464 1.9235	1.935 $\pm$ 0.016	0.8	100	0
0.5 ppb Calibrator	1.6771 1.6877	1.682 $\pm$ 0.007	0.5	87	1.25
2 ppb Calibrator	1.1403 1.2411	1.206 $\pm$ 0.057	4.7	62	5.0
5 ppb Calibrator	0.9823 0.9802	0.981 $\pm$ 0.01	0.1	51	12.5
8 ppb Calibrator	0.8121 0.8206	0.811 $\pm$ 0.011	1.3	42	20
32 ppb Calibrator	0.4512 0.4442	0.449 $\pm$ 0.004	0.9	23	80

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

\* standard deviation

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

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## TECHNICAL ASSISTANCE

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

## 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

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