



## Ivermectin Tube Kit

**Cat. # 20-0285**

(FOR ANALYSIS OF PLASMA SAMPLES)

Product Insert

READ COMPLETELY BEFORE USE.

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### INTENDED USE

The Beacon Ivermectin Tube Kit is a competitive ELISA for the qualitative analysis of Ivermectin and related compounds in plasma samples.

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### USE PRINCIPLES

The Beacon Ivermectin tube kit is a competitive enzyme-labeled immunoassay. Ivermectin HRP enzyme conjugate is pipetted into the test tubes followed by controls or sample extract. An Ivermectin antibody solution is then added into the test tubes 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 tubes 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 tube 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 will expire the last day of the month indicated on the box label when stored at 2 to 8°C.

- **40 Antibody Coated Tubes** vacuum-packed in aluminized pouch with indicating desiccant
- **Extraction Buffer** – (1) vial containing 6 mL
- **Dilution Buffer for Tube assay** – (1) bottle containing 30 mL (Caution! Contains organic solvent.)
- **Negative Control** – (1) vial containing 10 mL
- **Positive Control** – (1) vial containing 10 mL
- **Ivermectin Antibody Solution** – (1) bottle containing 24 mL

- **Ivermectin-HRP Enzyme Conjugate** – (2) vials each containing 11 mL
- **Substrate** – (1) bottle containing 24 mL
- **Stop Solution** – (1) bottle containing 24 mL (Caution! Contains 1N HCl. Handle with care.)
- **Wash Solution salts** – (1) packet

#### **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 Vacutainer K2 EDTA tube (BD Cat. # 367899) or equivalent
- Pipettes with tips capable of dispensing 50 to 200 µL and 500 to 1000 µL
- Photometer capable of reading 12 mm tubes at 450 nm (HACH Pocket Colorimeter II) with tube adaptor
- Timer
- Vortex mixer
- Wash bottle
- Paper towels or equivalent absorbent material

#### **SPECIFICITY**

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

<b>Compound</b>	<b>% CR</b>
Ivermectin	100 %
Abamectin	160 %
Doramectin	37 %
Eprinomectin	141%
Avermectin B1a	167%
Avermectin B1b	109%

#### **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 Tube Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Ivermectin Tube Kits with different lot numbers. Do not use the dilution buffer from Beacon's Ivermectin HS Plate Kit (Cat.# 20-0272) in 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 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 properly sized and calibrated pipettes is critical to obtain proper assay results. Please pipette carefully.
- Keep Dilution Buffer bottles tightly capped when not in use to prevent evaporation of organic solvent in the solutions.

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## SAMPLE PREPARATION (EDTA plasma)

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** (at high speed).
8. **Let sample stand** until clear top layer (supernatant) appears. This will take approximately 30 seconds.
9. **Dilute** the clear top layer of sample (1:4) with Dilution Buffer (0.2 mL of supernatant + 0.6 mL of Dilution Buffer).
10. **Vortex for 30 seconds to mix** (at high speed).
11. Prepare sample immediately before analysis to minimize evaporation that can occur upon standing at room temperature.

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## ASSAY PROCEDURE

1. Allow reagents and sample extracts to reach room temperature prior to running the test.
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 working wash solution to a wash bottle.
3. Place a maximum of 8 tubes into a tube rack (A single run should always include 2 Controls (Negative & Positive) in addition to a maximum of 6 unknown plasma samples). Label tubes with samples or control level. Securely seal unused tubes in the aluminized pouch bag with desiccant.
4. **Dispense 500 µL of the Enzyme Conjugate** into each test tube.
5. **Add 500 µL of the \*Negative Control, \*Positive Control or \*Sample Extract** into the appropriate tubes using a clean pipette tip for each.
6. **Dispense 500 µL of the Antibody Solution** into each test tube.
7. Shake the tube rack for 30 seconds to mix contents. The tube rack should remain on table and move right to left to mix contents gently (Do not lift rack and shake).
8. **Incubate for 30 minutes** at room temperature.
9. **Wash 4 times** by decanting all of the tube contents into an appropriate waste container and filling tubes to overflowing 4 times with working Wash Solution.
10. Following the last wash, tap the inverted tubes several times onto absorbent paper towels to remove the last of the wash solution
11. **Dispense 500 µL of the Substrate** into each tube.
12. **Shake** the tube rack for 30 seconds as described in step 7.
13. **Incubate for 30 minutes** at room temperature.
14. **Dispense 500 µL of the Stop Solution** into each tube. Shake the tube rack gently to mix.
15. Measure and record the absorbance (Optical Density; OD) of the tubes at 450 nm using the HACH Pocket Colorimeter II.

*\*Keep vials containing controls (negative & positive) and sample extracts tightly capped when not in use to prevent evaporation.*

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## CALCULATION OF RESULTS

Qualitative results can be derived by comparing the %B/B0 value of Positive Control to the Sample %B/B0.

- **To calculate the %B/B0 of the Positive Control**  
Divide OD of Positive Control by OD of Negative Control X100.
- **To calculate the %B/B0 of the Sample**  
Divide OD of Sample by OD of Negative Control X 100.

## INTERPRETATION OF RESULTS

- A %B/B0 result from a Sample less than the %B/B0 value of the Positive Control is expected to contain a concentration greater than or equal to 10 ppb Ivermectin. The sample is positive and should be tested by a confirmatory method such as the Beacon Ivermectin HS Plate Kit (#20-0272).
- A %B/B0 result from a Sample greater than the %B/B0 value of the Positive Control is expected to contain a concentration of less than 10 ppb Ivermectin. This sample is considered negative for this assay.

## SAMPLE CALCULATION

Sample	OD value	%B/B0	Score (P/N)
Negative Control	1.233	100	Negative
Positive Control	0.802	65	Reference
Sample #1	0.715	58	10 ppb or greater
Sample #2	0.850	69	Less than 10 ppb

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## 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

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