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User's Manual

High Sensitivity Ivermectin ELISA Kit

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Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The High Sensitivity Ivermectin ELISA Kit is a competitive ELISA for the quantitative analysis of ivermectin and related compounds in plasma samples.

Principles of Testing

The High Sensitivity Ivermectin ELISA Kit is a 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 enzyme conjugate compete for binding to the ivermectin antibody. Following this incubation, the wells are washed to remove any unbound ivermectin and ivermectin HRP enzyme 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 a final 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.

Reagents And Materials Provided

1. Plate containing 12 test strips of 8 wells each vacuum-sealed in an aluminized pouch with a desiccant.
2. 4 Vials Ivermectin Calibrators (0, 0.1, 0.6, 3.6 ppb)
3. 1 Bottle of Ivermectin HRP Enzyme Conjugate
4. 1 Bottle of Ivermectin Antibody
5. 1 Bottle of Dilution Buffer (Caution! Contains organic solvent)
6. 1 Packet of Wash Solution
7. 1 Bottle of Substrate
8. 1 Bottle of Stop Solution (Caution! Contains 1N HCl. Handle with care.)

Materials Required But Not Supplied

1. Laboratory quality distilled or deionized water
2. Acetonitrile (ACS grade)
3. Sample extraction or dilution tube (culture tube 12 X 75 mm or equivalent
4. Becton, Dickinson and Company (BD) Vacutainer K2 blood collection EDTA tube or equivalent
5. Pipette capable of dispensing 50 to 200 µL
6. Pipette capable of dispensing 500 to 1000 µL
7. Multi-channel pipette; 8 channels capable of dispensing 50 µL
8. Paper towels or equivalent absorbent material

9. Micro well plate or strip reader with 450 nm filter
10. Orbital shaker (optional)
11. Timer
12. Vortex mixer
13. Wash bottle

Storage

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.

Specimen Collection And 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. 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.
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 ziplock bag with desiccant.
4. Dispense **50 µL of HRP Enzyme Conjugate** into each well.

5. Dispense **50 µL of Calibrators or Sample Extract** to the appropriate wells. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
6. Dispense **50 µL of Antibody** into each well. If running more than two strips at once, the use of a multichannel pipette is recommended.
7. Gently shake the plate for 30 seconds using a back-and-forth motion and 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 this wash step four times for a total of five washes. Following the last wash, tap the inverted wells onto absorbent paper to remove excess wash solution.
9. Dispense **100 µL of Substrate** into each well.
10. Incubate for **30 minutes** at room temperature.
11. Dispense **100 µL of Stop Solution** into each well in the same order of addition as the Substrate. If running more than two strips at once, the use of a multi-channel pipette is recommended.
12. Measure and record the absorbance (Optical Density; OD) of the wells at 450 nm using a strip or plate reader. If the reader has dual wavelength capability, read at 450 nm minus 605 or 650 nm.
13. To obtain the plasma concentrations of ivermectin, multiply the results by a factor of 15.

Interpretation Of Results

Semi-Quantitative Interpretation: Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrator wells: Samples with a lower absorbance (less color) than a calibrator well have a concentration of ivermectin greater than the concentration of the calibrator. Samples with a higher absorbance (more color) than a calibrator well have a concentration less than the concentration of the calibrator.

Quantitative Interpretation: It is preferred for quantitative results to be determined using commercially available software for ELISA evaluation using 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 54 ppb, respectively. Samples with absorbances lower than the highest calibrator contain a concentration of ivermectin too high for quantification. Further dilute the sample extract in laboratory quality distilled or deionized water and retest along with the calibrators and control. Samples should be diluted to fit into the standard curve. Results must then be multiplied by the dilution factor used.

Typical Standard Curve

Well Contents (ppb)	OD		Average OD ± SD*	%RSD	$\%B_o$**	Plasma Conc. (ppb)
0	1.850	1.909	1.879 ± 0.041	2.2	100	0
0.1	1.640	1.592	1.616 ± 0.034	2.1	86	1.5
0.6	1.036	1.032	1.034 ± 0.003	0.3	55	9
3.6	0.459	0.440	0.449 ± 0.013	3.0	24	54

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

* standard deviation

** $\%B_0$ = (Average OD / 0 ppb Average OD)*100

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

1. Store all kit components at 4°C to 8°C (39°F to 46°F) when not in use.
2. Each reagent is optimized for use in the Ivermectin Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Ivermectin Plate Kits with different lot numbers.
3. Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
4. Do not use reagents after expiration date.
5. Reagents should be brought to room temperature, 20 to 28°C (62 to 82ºF) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
6. Ivermectin is a toxic antiparasitic drug and should be treated with care.
7. 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.
8. Transfer of samples and reagents by pipette requires constant monitoring of technique. Pipetting errors are the major source of error in immunoassay methodology.
9. Keep Calibrators and Dilution Buffer bottles tightly capped when not in use to prevent evaporation of organic solvent in the solutions.
10. If running more than two strips at once, the use of a multichannel pipette is required.