Avermectin and Ivermectin ELISA Kit

AVM & IVM ELISA

Product #: E6009-96T

Manufacturer Information

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1. Description

The avermectins are a series of drugs and pesticides used to treat parasitic worms and insect pests. They are a group of 16-membered macrocyclic lactone derivatives with potent anthelmintic and insecticidal properties. The macrocyclic lactones (MLs) are an important class of chemicals, which are used worldwide as veterinary drugs and as crop protection agents. As a result, residues of MLs are important from both a food safety and environmental perspective. This ELISA Kit is based on indirect competitive ELISA to detect avermectin residue in milk and tissue.

2. Application

This kit is applicable for determination of avermectin in milk and tissue.

3. Kit components

1) Microtiter plate, 96wells, 1 plate
2) Avermectin standards, 1mL/vial, 6 vials, 0, 1, 3, 9, 27, 81 ppb
3) High concentration standard, 1mL/vial, 1 vial, 1ppm
4) Enzyme conjugate, 1mL, with red cap
5) Antibody Solution, 10mL, with green cap
6) TMB A Substrate, 7mL, with white cap
7) TMB B Substrate, 7mL, with red cap
8) Stop solution, 7mL, with yellow cap
9) 20x Wash buffer, 40mL, with transparent cap
10) 2x Sample buffer, 50mL, with blue cap

4. Instrument and material required

1) ELISA reader, with 450/630nm
2) Centrifuge
3) Homogenizer
4) Balance, 0.01g
5) Centrifuge tube, 2mL, 10mL
6) Vortex mixer
7) Micropipette, 20-200μL, 100-1000μL
8) Multi-channel pipette, 250μL
9) graduated pipette,10mL

5. Reagent required

Deionized water
Acetonitrile (AR)
Methanol (AR)
n-Hexane (AR)
Neutral alumina (AR)

6. Buffer preparation

Buffer 1 Wash buffer:

Dilute 20x wash buffer with deionized water, in the volume ratio of 1:19, fox example, 10mL 20xwash buffer + 190mL deionized water, mix thoroughly.

This diluted wash buffer can be stored at 4°C for 1 month.

Buffer 2 Sample buffer:

For milk and tissue testing: dilute 2x sample buffer with deionized water, in the volume ratio of 1:1,

fox example, 10mL 2x sample buffer + 10mL deionized water, mix thoroughly.
This diluted wash buffer can be stored at 4°C for 1 month.

7. Sample preparation

7.1 Precautions before prepare samples:

1) Use disposable tips during the test. Change new tip for different sample / reagent.
2) Make sure all lab wares are clean and ready to use.
3) Prepared sample shall be analyzed immediately after dilution.

7.2 Milk

Take 2mL milk sample into a 50mL tube, add 4mL acetonitrile and 1mL n-hexane, then add 1g of neutral alumina, vortex for 5min, and then centrifuge for 5min at 3000g; remove the up layer n-hexane, and then take 1mL clear liquid into a 10mL tube, dry it under nitrogen at water bath (50-60°C). Add 100μL of methanol, and vortex for 30s, then add 900μL diluted sample buffer, vortex for 30s to mix thoroughly, and then take 50μL for assay.

7.3 Tissue

Weigh 4±0.05g homogeneous tissue sample into a 50mL tube, add 4mL methanol, then vortex for 5min; centrifuge for 5min at 3000g; take 2ml of supernate into a 10ml centrifuge tube, add 3mL acetonitrile and 1mL n-hexane, then add 1g of neutral alumina, vortex for 1min, and then centrifuge for 5min at 3000g; remove the up layer n-hexane, and then take 1mL clear liquid into a 10mL tube, dry it under nitrogen at water bath (50-60°C). Add 100μL of methanol, and vortex for 30s, then add 900μL diluted sample buffer, vortex for 30s to mix thoroughly, and then take 50μL for assay.

8. Notice and precautions before assay

1) Make sure the ELISA kit and all reagents are returned to room temperature (20-25 °C). For example, keep these reagent and kits at room temperature for at least 60min.
2) Return unused kit components to 2-8 °C.
3) Washing step is important for the reproducibility of the kit, please follow this instruction carefμLly.
4) Cover the ELISA plate during all incubation. Avoid direct sunlight.

9. Assay procedures

1) Return the ELISA kit and all reagents to room temperature (20-25 °C). For example, keep these reagent and kits at room temperature for at least 60min.
2) Take needed microwells and zip rest in the zip-bag and return to 2-8 °C.
3) Layout the plate and record sample and standard well positions. It is recommended to run all tests in duplicates.
4) **Add sample/standard/antibody**: add sample/standard into the wells, 50μL per each.
5) **Prepare the antibody solution and enzyme conjugate mixture**: mix the antibody solution and enzyme conjugate in the ratio of 10:1.
6) **Add antibody solution and enzyme conjugate mixture**: add the mixture into the wells, 50μL per each, shake gently and then cover the plate and incubate at 25 °C for 30min, avoid sunlight.
7) **Wash**: take out the plate and pour the liquid out. Use the diluted wash buffer (buffer 1) to wash the plate, 250μL /well. Wash for 4-5 times with interval of 10s. The pour the liquid out and tap the plate against absorbent paper. Eliminate the air bubble in the wells with

micropipette tip if the bubble exists.

8) **Coloration**: add TMB A substrate, 50μL per well, then add TMB B substrate, 50μL per well, and then cover the plate and incubate **25 °C for 15min,** avoid sunlight.
9) **Stop the reaction**: add stop solution, 50μL per well, shake gently and read the plate with ELISA reader at 450nm. Read the plate within 5min after adding stop solution.

10. Result Calculation

10.1 Qualitative estimation

This kit is based on competitive ELISA, thus the OD values is inversely proportional to the avermectin content contained in sample. If there is no ELISA reader, just compare the color of sample with the avermectin Standard to get the estimated sample avermectin content.

10.2 Quantitative calculation

With ELISA reader, a standard curve can be plotted with the ODs obtained. Use Logit-log, Cubic spline or logistic curve, etc to calculate the avermectin sample content.

Usually these software will be installed with your ELISA reader. If it is not provided, please contact us for help, spreadsheet with Logit-log calculation will be provided upon your request.

$$\text{OD Percentage } (\%) = \frac{B}{B0}$$

B – mean OD of standards or samples

B0 – mean OD of first standard (0 ng/L)

11. Sample dilution factor:

Tissue: 5 times;

Milk: 3 times.

12. Specifications of the kit

1) Sensitivity: 1ppb
2) Specificity: 200% to avermectin, 100% to ivermectin, 50% to doramectin
3) Limit of Detection:
   Tissue: ivermectin: 5ppb; avermectin: 2.5ppb;
   Milk: ivermectin: 3ppb; avermectin: 1.5ppb
4) Recovery: 90%±20%
5) Precision: C.V<10%.

13. Cautions and tips for the test

1) Lower room temperature, e.g., lower than 20◦C may cause lower OD values. Please make sure all reagent and kit components are returned to room temperature.
2) Wash step is vital for the reproducibility of the kit. Please wash according to the kit instruction. Do not let the plate dry during wash. Continue the next operations immediately after wash step.
3) Shake each reagent gently before use.
4) Stop solution is acidic, please handle with care.
5) Do not use expired kits and reagents. Do not mix the reagent and kits from different LOT.
6) The kit is stored at 2-8◦C(36-46◦F), do not freeze.
7) TMB substrate is sensitive to sunlight. Avoid direct sunlight.
8) If Standard 1 (0ng/L) OD is lower than 0.5, please do not use. The kit may be expired or deteriorated.

9) The coloration step takes 15min. You can prolong it to 20min-25min if the color of the well is too light. On the contrary, please reduce the incubation time.
10) The incubation is 37 ◦F, lower or higher temperature will cause changes of OD and sensitivity of the kits, which may affect the result of the assay.

14. Storage and expiration

The kit is valid for 12 months when stored at 2-8 ◦C. LOT and Expiry information are printed on the package.