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Avermectin ELISA Kit

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(Catalog # E4944-100, 96 assays, Store at 4°C)

I. Introduction:

Avermectin is a 16-membered macrocyclic lactone derivative that is produced as a fermented product by the soil-dwelling actinomycete *Streptomyces avermitilis*. Due to its anthelmintic and insecticidal properties, it is widely used to treat parasitic infections in humans and animals and also used as a pesticide in agriculture to prevent insect attacks on crops. The mode of action of the drug is that it binds to and opens the glutamine gated chloride channel on the invertebrate muscle cells and neurons. This causes an increased influx of chloride ions into the cells which lead to hyperpolarization of the membranes, and ultimately resulting in paralysis and death of the invertebrates. BioVision's Avermectin ELISA kit is used to quantitatively measure Avermectin in tissue and milk samples. The kit is based on the Competitive ELISA principle. Samples and standards are added to the microwell plate that is pre-coated with an antigen and competes for binding to the anti-Avermectin antibody. The HRP conjugate is added to each well and any unattached conjugates are washed off using Wash Buffer. The HRP enzymatic reaction is detected by the addition of substrate reagents. Finally, the reaction is terminated with an acidic stop solution. The color developed is inversely proportional to the concentration of Avermectin in the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Avermectin
Detection Limit: 4ppb for tissues; 5ppb for milk
Sensitivity: 0.5ppb
Cross reaction: Avermectin 100%, Ivermectin 25%, Eprinomectin 10%, Doramectin 6%

III. Sample Type:

Tissue (Chicken, Pork, Duck), milk

IV. Kit Contents:

Components	E4944-100	Part No.
Micro ELISA Plate	8 X 12 Strips	E4944-100-1
Standard (S0 – S5)	1 ml X 6	E4944-100-2
Enzyme Conjugate (11X)	0.7 ml	E4944-100-3
Enzyme Conjugate dilution	7 ml	E4944-100-4
Substrate A	7 ml	E4944-100-5
Substrate B	7 ml	E4944-100-6
Stop Solution	7 ml	E4944-100-7
Wash Buffer (20X)	30 ml	E4944-100-8
Redissolving Solution	50 ml	E4944-100-9
Sample Treatment Solution	10 ml	E4944-100-10
Plate Sealer	3	E4944-100-11

V. User Supplied Reagents and Equipment:

- Chemicals: deionized water, Methanol
- Microplate reader capable of measuring absorbance at 450 nm
- Clean eppendorf tubes and graduated cylinders for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment. The opened kit may be stable for 1 month at 4°C.

VII. Reagent and Sample Preparation:

Note: Bring all reagents to room temperature (20-25°C) 30 minutes before use.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Wash Buffer (1X):** Dilute 15 mL of **Wash buffer (20X)** with deionized water to 300 ml

2. **Enzyme Conjugate (1X):** Take 1 part of **Enzyme Conjugate (11X)** and dissolve it in 10 parts of **Enzyme Conjugate dilution** to prepare Enzyme conjugate (1X) solution. This diluted solution can be used for the assay.

3. **Standards Preparation:** Ready-to-use standards are as follows

Standards	S0	S1	S2	S3	S4	S5
Conc. (ppb)	0	0.5	1.5	4.5	13.5	40.5

4. **Sample Preparation:**

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Note: The prepared sample maybe stored for up to one day at 2-8°C.

Sample pre-treatment: The following method must be used for pre-treatment of any kind of sample:
Note: Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents.

Sample Preparation and pre-treatment (for Tissue samples):

Detection limit: 4ppb

- Take 2 ± 0.05 grams of the homogenized tissue sample into a 10 ml centrifuge tube. Add 2 ml **Methanol**, 100 µl **Sample treatment solution**. Vortex for 5 mins until the separation of tissue samples, centrifuge at 4000 x g for 10 mins at room temperature (RT) (25 ± 2 ^{0}C).
- Take 200 µl of the clear liquid (upper layer) and add 400 µl of **Sample redissolving solution**. Vortex for 10 secs.
- Take 50 µl for the analysis
- **Fold of dilution of the sample: 6**

Sample Preparation and pre-treatment (for Milk):

Detection limit: 5ppb

- Take 1 ml of milk sample into a 10 ml centrifuge tube. Add 3 ml **Methanol**. Vortex for 1 min, centrifuge at 4000 x g for 10 mins at room temperature (RT) (25 ± 2 ^{0}C).
- Take 200 µl of the clear liquid (upper layer) and add 400 µl of **Sample redissolving solution**. Vortex for 10 secs.
- Take 50 µl for the analysis
- **Fold of dilution of the sample: 10**

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Add 50 µl of the **sample or standards** to separate duplicate wells. Add 50 µl of the **Enzyme Conjugate (1X)** into each well. Mix gently by shaking the plate manually, seal the microplate with the plate sealer, and incubate in dark at 25 ^{0}C for 30 mins.
2. Remove the plate sealer carefully, aspirate liquid out of microwells, and add 300 µl of **Wash Buffer (1X)** to each well. Wash for 15-30 secs, and then discard the buffer. Repeat the washing step four to five times. After the final wash step, tap to dry (if there are the bubbles after tapping, remove them with the clean tips).
3. Add 50 µl of the **Substrate A** and then 50 µl of the **Substrate B** into each well. Mix gently by shaking the plate manually, and incubate at 25 ^{0}C for 15 mins at dark.
4. Add 50 µl of the **Stop Solution** into each well. Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450 nm to determine the OD value (Recommend reading the OD value at the wavelength 450 nm within 5 mins).

IX. Calculation:

- **Quantitative determination**

The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

Absorbance Value (%) = B/B_0 X 100%

B: The average absorbance value of the sample or standard

B_0 : The average absorbance value of the 0 ppb standard

To draw a standard curve: Take the absorbency value of standards as y-axis, logarithmic of the concentration of the Avermectin standards solution (ppb) as x-axis. The Avermectin concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

X. Related Products:

Streptomycin ELISA Kit (E4272)
Gentamicin (serum/urine) ELISA Kit (K4315)
Kanamycin ELISA Kit (K4210)
Quinolone ELISA Kit (E4530)
Tylosin ELISA Kit (E4779)

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