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Doramectin ELISA Kit

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(Catalog # E4958-100, 96 assays, Store at 4°C)

I. Introduction:

Doramectin is a macrocyclic lactone obtained from the fermentation of soil organism *Streptomyces avermitilis*. It belongs to the class of Avermectins and is structurally similar to Ivermectin. Due to its anthelmintic and insecticidal properties, it is widely used to treat parasitic infections in 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. Doramectin is prohibited to be used in dairy-producing animals as the accumulation of the drug in milk residues may pose a health risk in humans. BioVision's Doramectin ELISA kit is used to quantitatively measure Doramectin in milk and yogurt 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-Doramectin 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 Doramectin in the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Doramectin
Detection Limit: 1ppb for yogurt, raw and finished milk
Sensitivity: 0.1ppb
Cross reaction: Doramectin 100%, Abamectin 300%, Ivermectin 227%, Eprinomectin 300%

III. Sample Type:

Raw milk, Finished milk, Yogurt

IV. Kit Contents:

Components	E4958-100	Part No.
Micro ELISA Plate	8 X 12 Strips	E4958-100-1
Standard (S0 – S5)	1 ml X 6	E4958-100-2
HRP Conjugate	12 ml	E4958-100-3
Antibody working solution	7 ml	E4958-100-4
Milk Precipitant	10 ml	E4958-100-5
Substrate A	6 ml	E4958-100-6
Substrate B	6 ml	E4958-100-7
Stop Solution	6 ml	E4958-100-8
Wash Buffer (20X)	25 ml	E4958-100-9
Sample Diluent A	20 ml	E4958-100-10
Sample Diluent B	20 ml	E4958-100-11
Plate Sealer	3	E4958-100-12

V. User Supplied Reagents and Equipment:

- Chemicals: deionized water, Methanol
- Microplate reader
- 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. 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 1 part of **Wash buffer (20X)** with 19 parts of deionized water. Prepare quantity as needed.

2. **Standards Preparation:** Ready-to-use standards provided as follows

Standards	S0	S1	S2	S3	S4	S5
Conc. (ppb)	0	0.1	0.2	0.4	0.8	1.6

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

FOR RESEARCH USE ONLY! Not to be used on humans.

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 raw and finished milk samples):

Detection limit: 1ppb

- Take 2 ml of the fresh raw or finished milk sample into a 10 ml centrifuge tube. Add 100 µl of the **Milk Precipitant** and then add 3 ml of **Methanol**. Mix well. Centrifuge at 4000 r/min for 5 mins
- Take 0.2 ml of the supernatant in a new centrifuge tube and add 0.2 ml of **Sample Diluent A**. Mix for 30 secs.
- Take 50 µl of the supernatant for the analysis.
- **Fold of dilution of the sample: 5**

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

Detection limit: 1ppb

- Take 2 ml of the fresh yogurt sample into a 10 ml centrifuge tube. Add 3 ml of Methanol**.** Mix for 1 min. Centrifuge at 4000 r/min for 5 mins.
- Take 0.2 ml of the supernatant in a new centrifuge tube and add 0.2 ml of **Sample Diluent B**. Mix for 30 secs.
- Take 50 µl of the supernatant for the analysis.
- **Fold of dilution of the sample: 5**

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 **Antibody working solution** into each well. Mix gently for 10 secs by shaking the plate manually, seal the microplate with the plate sealer, and incubate in dark at 25 ^{0}C for 30 minutes in the dark.
2. Remove the plate sealer carefully, aspirate liquid out of microwells, and add 260 µl of **Wash Buffer (1X)** to each well. Wash for 30 secs, and then discard the buffer. Repeat the washing step 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 100 µl of **HRP Conjugate** in each well and incubate at 25 ^{0}C for 30 mins in dark.
4. **Repeat washing as in step 2.**
5. Add 50 µl of the **Substrate A** and then add 50 µl of the **Substrate B** into each well. Mix gently for 5 secs by shaking the plate manually, and incubate at 25 ^{0}C for 15 mins in dark.
6. Add 50 µl of the **Stop Solution** into each well. Mix gently for 10 secs 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: Plot the absorbance value of standards as y-axis, logarithmic of the concentration of the Ribavirin standards solution (ppb) as x-axis. The Ribavirin 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:

Sulfamethazine ELISA Kit (E4778)
Norfloxacin ELISA Kit (E4776)
Chlortetracycline ELISA Kit (E4782)
Ampicillin ELISA Kit (E4350)
Salbutamol (SALB) ELISA Kit (K4209)