Creative Diagnostics®

Version 10-05/25

User's Manual

Avermectins/Ivermectin ELISA Kit

REF **DEIA-CL015**

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.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The Avermectins/Ivermectin ELISA Test Kit is a competitive enzyme immunoassay for the quantitative analysis of Abamectin in meat, animal tissue, and milk.

General Description

The method is based on a competitive colorimetric ELISA assay. The drug of interest has been coated in the plate wells. During the analysis, sample is added along with the primary antibody specific for the target drug. If the target is present in the sample, it will compete for the antibody, thereby preventing the antibody from binding to the drug attached to the well. The secondary antibody, tagged with a peroxidase enzyme, targets the primary antibody that is complexed to the drug coated on the plate wells. The resulting color intensity, after addition of substrate, has an inverse relationship with the target concentration in the sample.

Reagents And Materials Provided

Avermectin-coated Microtiter Plate 1 x 96-well plate (8 wells x 12 strips)

Avermectin Standards:

Negative control

0. 125 ng/mL 1.0ml

0. 25 ng/mL 1.0ml

0. 5 ng/mL 1.0ml

1 ng/mL 1.0ml

2 ng/mL 1.oml

100 ng/mL (spiking, optional) 1.0ml

Avermectin Antibody #1 6 ml

HRP-Conjugated Antibody #2 11 ml

20X Wash Solution 30mL

Stop Buffer 11 mL

TMB Substrate 11 mL

10X Sample Extraction Buffer 15mL

Materials Required But Not Supplied

Microtiter plate reader (450 nm)

Incubator

Tissue Mixer (e.g. Omni TissueMaster Homogenizer)

Vortex mixer (e.g. Gneie Vortex mixer from VWR)

10, 20, 100 and 1000 uL pipettes

Multi-channel pipette: 50-300 uL (Optional)

Acetonitrile

Ethanol

n-hexane

MgSO4

Storage

Store the kit at 2-8°C *. The shelf life is 12 months when the kit is properly stored.

Specimen Collection And Preparation

Be sure samples are properly stored. In general, samples should be refrigerated at 2-4°C for no more than 1-2 days. Freeze samples to a minimum of -20°C if they need to be stored for a longer period. Frozen samples can be thawed at room temps (20 – 25°C / 68 – 77°C) or in a refrigerator before use.

1. Preparation of 1X Sample Extraction Buffer:

Mix 1 volume of 10X Sample Extraction Buffer with 9 volumes of distilled water.

2. Preparation of 25% Ethanol/ Sample Extraction Buffer

Mix 7.5 volumes of 1X Sample Extraction Buffer with 2.5 volumes of 100% ethanol

Meat/Fish/Shrimp

1. Homogenize a reasonable amount of sample with a suitable mixer.
2. Weigh out 2 g of the homogenized sample. Add 6 mL of acetonitrile, 2 mL of n-hexane, and 1 g of anhydrous MgSO4, vortex 3 min at maximum speed and then at rotorack for 15 minutes.
3. Centrifuge for 5 minutes at 6000 x g at room temperature (20 – 25°C / 68 – 77°F).
4. Take 400 uL of supernatant to a 2 mL tube and use an evaporator to dry the sample at 50°C under reduced pressure. Alternatively, the sample can be dried by blowing nitrogen gas in a 50°C waterbath.
5. Add 500 uL of 25%Ethanol/1x Sample Extraction Buffer to the dried tube and vortex vigorously for 2 minutes.
6. Use 50 uL of the suspension per well in the ELISA test.

Note: Dilution factor: 5

Milk

1. Take 0.5 mL of milk sample, add 625uL of ethanol, shake 5 seconds and 1.375mL of 1X Sample Extraction Buffer, shake 30 seconds.
2. Centrifuge at 8,000 x g for 5 minutes.
3. Use 50uL of the sample per well for the assay.

Note: Dilution factor: 5

Reagent Preparation

IMPORTANT: All reagents should be brought up to room temperature before use (1 – 2 hours at 20 – 25°C / 68 – 77°F); Make sure you read "Warnings and Precautions" section on page 3. Solutions should be prepared just prior to ELISA test. All reagents should be mixed by gently inverting or swirling prior to use. Prepare volumes that are needed for the number of wells being run. Do not return the reagents to the original stock tubes/bottles. Using disposable reservoirs when handling reagents can minimize the risk of contamination and is recommended.

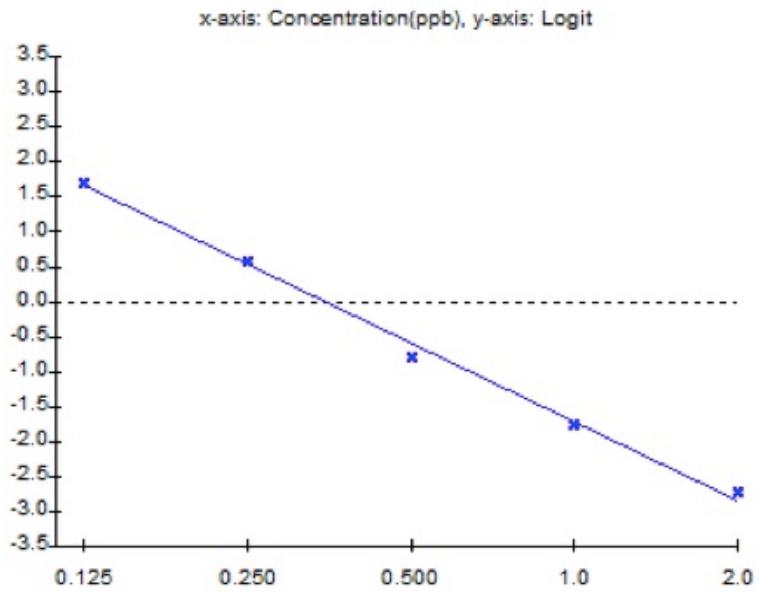
1. Preparation of 1X Wash Solution

Mix 1 volume of the 20X Wash Solution with 19 volumes of distilled water.

Assay Procedure

1. Add 50 uL of each Avermectin Standard in duplicate into different wells (Add standards to plate only in the order from low concentration to high concentration).
2. Add 50 uL of each sample in duplicate into different sample wells.
3. Add 50 uL of Avermectin Antibody #1 to each well and mix well by gently rocking the plate manually for 1 minute.
4. Incubate the plate for 30 minutes at room temperature (20 – 25°C. / 68 – 77°F.).
5. Wash the plate 4 times with 250 uL of 1X Wash Solution. After the last wash, invert the plate and gently tap the plate dry on paper towels (Perform the next step immediately after plate washings. Do not allow the plate to air dry between working steps).
6. Add 100uL of HRP-Conjugated Antibody #2. Incubate the plate for 30 minutes at room temperature (20 – 25°C. / 68 – 77°F.) (Avoid direct sunlight and cold bench tops during the incubation. Covering the microtiter plate while incubating is recommended).
7. Wash the plate 4 times with 250 uL of 1X Wash Solution. After the last wash, invert the plate and gently tap the plate dry on paper towels (Perform the next step immediately after plate washings. Do not allow the plate to air dry between working steps).
8. Add 100 uL of TMB substrate. Time the reaction immediately after adding the substrate. Mix the solution by gently rocking the plate manually for 1 minute while incubating (Do not put any substrate back to the original container to avoid any potential contamination. Any substrate solution exhibiting coloration is indicative of deterioration and should be discarded. Covering the microtiter plate while incubating is recommended).
9. After incubating for 15 minutes at room temperature (20 – 25°C. / 68 – 77°F.), add 100 uL of Stop Buffer to stop the enzyme reaction.
10. Read the plate as soon as possible following the addition of Stop Buffer on a plate reader with 450 nm wavelength (Before reading, use a lint-free wipe on the bottom of the plate to ensure no moisture or fingerprints interfere with the readings).

Typical Standard Curve



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