Version 01-05/24

User's Manual

Albendazole Elisa Kit

REF **DEIANJ15**

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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

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

Albendazole ELISA Kit is a competitive enzyme immunoassay for the quantitative analysis of Albendazole in meat and feed.

General Description

Albendazole, also known as albendazolum, is a medication used for the treatment of a variety of parasitic worm infestations. It is useful for giardiasis, trichuriasis, filariasis, neurocysticercosis, hydatid disease, pinworm disease, and ascariasis, among others.

Common side effects include nausea, abdominal pains, and headaches. Potentially serious side effects include bone marrow suppression which usually improves on stopping the medication. Liver inflammation has been reported and those with prior liver problems are at greater risk. It is pregnancy category C in the United States and category D in Australia, meaning it may cause harm if taken by pregnant women. Albendazole is a broad-spectrum antihelminthic agent of the benzimidazole type.

Principles of Testing

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

Albendazole Standards:

Negative control (white cap tube) 1ml

0. 15 ng/mL (yellow cap tube) 1ml
0. 45 ng/mL (orange cap tube) 1ml
1. 5 ng/mL (pink cap tube) 1ml
4. 5 ng/mL (purple cap tube) 1ml
13. 5 ng/mL (blue cap tube) 1ml

100 ng/mL (spiking, optional,red cap tube) 1ml

Albendazole Antibody #1 6 mL

ABD Clean Up Reagent 25 g

Concentrate of ABD Extraction Buffer 30g

100 X HRP-Conjugated Antibody #2 250 µL

Antibody #2 Diluent ** 20 mL

10X Sample Extraction Buffer F 25 mL

20X Wash Solution ** 28 mL

Stop Buffer ** 14 mL

TMB Substrate ** 12 mL

** If the kit is to be stored for over two weeks before use, Albendazole Antibody #1, 100X HRP-Conjugated Antibody #2 at -20°C in a freezer.

Materials Required But Not Supplied

Microtiter plate reader (450 nm)

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

10, 20, 100 and 1000 µL pipettes

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

Storage

Store the kit at 2 - 8°C.

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) or in a refrigerator before use.

1. Preparation of 1× Sample Extraction Buffer F

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

2. Preparation of 1× ABD Extraction Buffer

Take all of the powder from the Concentrate of ABD Extraction Buffer bag to a 125-mL bottle, add 90 mL of distilled water, vortex or mix manually for 2 minutes.Leave the solution at room temperature for 20 minutes it is normal for small particles to remain seen in the bottle.

Meat

1. Add 1 mL of 1X ABD Extraction Buffer and 4 mL of Acetonitrile to 1 g of meat sample, vortex for 3minutes at maximum speed manually or 10 minutes using a multi-tube vortexer.
2. Centrifuge for 5 minutes at 4,000 × g at room temperature (20 - 25°C).
3. Transfer 1.6 mL of the Acetonitrile supernatant to another tube, add about 300 mg (280-320 mg) ABD Clean Up Reagent, vortex for 30 seconds at maximum speed, leave at room temperature at least 5 minutes.
4. Transfer 1.2 mL of the supernatant to another tube (corresponding to 0.3 g of the original sample) into a new vial and use a rotary evaporator to dry the sample in a 60-70°C water bath under reduced pressure. Alternatively, the sample can be dried by blowing nitrogen gas in a 60-70°C water bath.
5. Dissolve the dried residue in 0.3 mL of 1× Sample Extraction Buffer F by vortexing at maximum speed for 1

minute.

6. Use 50 µL of the sample per well for the assay.

Note: Dilution Factor: 1.

Feed

1. Add 2 mL of 1× ABD Extraction Buffer and 4 mL of Acetonitrile to 1 g of ground feed sample, vortex for 3 minutes at maximum speed manually or 10 minutes using a multi-tube vortexer.
2. Centrifuge for 5 minutes at 4,000 x g at room temperature (20 - 25°C)
3. Transfer 1.6 mL of the Acetonitrile supernatant to another tube, add about 300 mg (280-320 mg) ABD Clean Up Reagent, vortex for 30 seconds at maximum speed, leave at room temperature at least 5 minutes.
4. Transfer 1.0 mL of the supernatant to another tube (corresponding to 0.25 g of the original sample) into a new vial and use a rotary evaporator to dry the sample in a 60-70°C water bath under reduced pressure. Alternatively, the sample can be dried by blowing nitrogen gas in a 60-70°C water bath.
5. Dissolve the dried residue in 0.5 mL of 1× Sample Extraction Buffer F by vortexing at maximum speed for 1 minute.
6. Use 50 µL of the sample per well for the assay.

Note: Dilution Factor: 2.

Reagent Preparation

IMPORTANT: All reagents should be brought up to room temperature before use (1 - 2 hours at 20 - 25°C); Make sure you read "Warnings and Precautions" section. 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 1× HRP-Conjugated Antibody #2

Mix 1 volume of 100× HRP-Conjugated Antibody #2 with 99 volumes of Antibody #2 Diluent.

2. Preparation of 1× Wash Solution

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

Assay Procedure

1. Add 50 µL of each Albendazole Standards in duplicate into different wells (Add standards to plate only in the order from low concentration to high concentration).
2. Add 50 µL of each sample in duplicate into different sample wells.
3. Add 50 µL of Albendazole Antibody #1 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).
5. Wash the plate 3 times with 250 µL of 1× 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 150 µL of 1× Antibody #2 solution. Incubate the plate for 30 minutes at room temperature (20 - 25°C) (Avoid direct sunlight and cold bench tops during the incubation. Covering the microtiter plate while

incubating is recommended).

7. Wash the plate 3 times with 250 µL of 1× 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 µL 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), add 100 µL of Stop Buffer to stop the enzyme reaction.
10. Read the plate as soon as possible following the addition of Stop Buffer on a platereader 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).

Calculation

A standard curve can be constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ng/mL on a logarithmic curve.

Relative absorbance (%) = absorbance standard (or sample) x 100 / absorbance zero standard

Precision

Interplate and intraplate CV typically < 10%

Detection Range

meat 1ppb

feed 2ppb

Sensitivity

0.15 ppb

Specificity

Albendazole 100%

Mebendazole 100%

Fenbendazole 80%

Benomyl 75%

4' -Hydroxyfenbendazole 48%

Benzaldehyde <0.1%

Cambendazole <0.1%

Precautions

The standards contain Albendazole. Handle with care.

Do not use the kit past the expiration date.

Do not intermix reagents from different kits or lots except for components with the same part No's within their expiration dates. ANTIBODIES AND PLATES ARE KIT-AND LOT-SPECIFIC. Make sure that the antibody #2 and diluent are mixed in correct volumes.

Try to maintain a laboratory temperature of 20 - 25°C. Avoid running assays under or near air vents, as this may cause excessive cooling, heating and/or evaporation. Also, do not run assays in direct sunlight, as this may cause excessive heat and evaporation. Cold bench tops should be avoided by placing several layers of paper towel or some other insulation material under the assay plates during incubation.

Make sure you are using only distilled or deionized water since water quality is very important.

When pipetting samples or reagents into an empty microtiter plate, place the pipette tips in the lower corner of the well, making contact with the plastic.

Incubations of assay plates should be timed as precisely as possible. Be consistent when adding standards to the assay plate. Add your standards first and then your samples.

Add standards to plate only in the order from low concentration to high concentration as this will minimize the risk of compromising the standard curve.

Always refrigerate plates in sealed bags with a desiccant to maintain stability. Prevent condensation from forming on plates by allowing them equilibrate to room temperature (20 - 25°C) while in the packaging.