Maduramicin ELISA Kit

Cat. No.:DEIA6839
Pkg.Size:96T

Intended use
The Maduramicin ELISA Kit is suitable for the quantitative and/or qualitative screening of Maduramicin in animal feed and contaminated products.

General Description
Maduramicin (maduramycin) is an antiprotozoal agent used in veterinary medicine to prevent coccidiosis. It is a natural chemical compound first isolated from the actinomycete Actinomadura rubra.

Principle Of The Test
The test is a direct competitive ELISA based on the recognition of Maduramicin by specific antibodies. Maduramicin, when present in a sample, and a Maduramicin-HRP analogue compete for the binding sites of rabbit anti-Maduramicin antibodies in solution. The Maduramicin antibodies are then bound by a second antibody (donkey anti-rabbit) immobilized on the wells of the microtiter plate. After a washing step and addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of Maduramicin present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

Reagents And Materials Provided
1. Microtiter plate (12 X 8 strips) coated with a secondary antibody, in a resealable aluminum pouch
2. Maduramicin Calibrators/Standards : 0, 0.25, 0.50, 1.0, 2.5 ng/mL (ppb), 1 mL each
3. Antibody Solution (rabbit anti-Maduramicin), 6 mL
4. Maduramicin-HRP Conjugate Solution, 6 mL
5. Wash Solution (5X) Concentrate, 100 mL, must be diluted before use.
6. Sample Diluent, 2 bottles, 25 mL each
7. Substrate (Color) Solution (TMB), 16 mL
8. Stop Solution, 12 mL (handle with care)

Materials Required But Not Supplied
1. Micro-pipettes with disposable plastic tips (50-200 µL)
2. Multi-channel pipette (50-250 µL) or stepper pipette with disposable plastic tips (50-250 µL)
3. Microtiter plate reader (wave length 450 nm)
4. Overhead tube rotator
5. Vortex mixer
6. Deionized or distilled water
7. Acetone, reagent grade
8. Paper towels or equivalent absorbent material
9. Timer
10. Centrifuge capable of spinning at 3,000 x g
11. 15 mL conical tubes with caps
12. 4 mL glass vials with Teflon-lined caps
13. Analytical 3 place balance

**Storage**

The Maduramicin ELISA should be stored in the refrigerator (4–8°C). The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box.

**Specimen Collection And Handling**

Dog/Cat Food, Vitamin, and Rice Hull Extraction

Note: Dog or Cat Food which is in pressed pellet form must be ground into powder before extraction.

Samples should be analyzed immediately after extraction.

1. Weigh 1.0 g of feed, vitamin mix, or rice hulls into a 15 mL conical tube.
2. Add 5 mL of acetone.
3. Vortex for 30 seconds.
4. Mix using an overhead tube rotator for 15 minutes.
5. Centrifuge for 5 minutes at 3000 x g.
6. Dilute 40 µL of the supernatant solution into 920 µL of Sample Diluent (1:24 dilution). Vortex. This will then be analyzed as sample.

The Maduramicin concentration in the sample is determined by multiplying the ELISA result by a factor of 120. Highly contaminated samples, those outside of the calibration range of the assay, must be diluted further and re-analyzed.

**Plate Preparation**

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

Std 0-Std 4: Standards (0; 0.25; 0.50; 1.0; 2.5 ppb)
Samp1, Samp2, etc.: Samples

Figure 1.
Reagent Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. In order to equalize the incubation periods on the entire microtiter plate, a multi-channel pipette or a stepping pipette is recommended for adding the enzyme conjugate, antibody, substrate, and stop solutions. Please only use the reagents and standards from one package lot in one test, as they have been adjusted in combination.

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the aluminum pouch. The remaining strips are stored in the aluminum pouch and zip-locked closed. Store the remaining kit in the refrigerator (4-8°C).
3. The standard solutions, conjugate, antibody, substrate and stop solutions are ready to use and do not require any further dilutions.
4. Dilute the Wash Solution (5X) Concentrate at a ratio of 1:5. If using the entire bottle (100 mL), add to 400 mL of deionized or distilled water and mix thoroughly.
5. The stop solution must be handled with care as it contains diluted H2SO4.

Assay Steps

1. Add 50 µL of the calibrator/standard solutions or sample extracts into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.
2. Add 50 µL of enzyme conjugate solution to the individual wells successively using a multi-channel pipette or a stepping pipette.
3. Add 50 µL of antibody solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 60 seconds. Be careful not to spill the contents.
4. Incubate the strips for 60 minutes at room temperature.
5. Remove the covering and decant the contents of the wells into a sink. Wash the strips four times using the diluted washing buffer solution. Please use at least a volume of 250 µL of washing buffer for each well in each washing step. Remaining buffer in
the wells should be removed by patting the inverted plate dry on a stack of paper towels.

6. Add 150 µL of substrate (color) solution to the wells. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 60 seconds. Be careful not to spill the contents. Incubate the strips for 20 minutes at room temperature. Protect the strips from direct sunlight.

7. Add 100 µL of stop solution to the wells in the same sequence as for the substrate solution.

8. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of stopping solution.

**Typical Standard Curve**

![Typical Standard Curve](image)

**Evaluation**

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs such as 4- Parameter (preferred) or Logit/Log. For a manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the %B/B0 for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the %B/B0 for each standard on the vertical linear (y) axis versus the corresponding Maduramicin concentration on the horizontal logarithmic (x) axis on graph paper. %B/B0 for samples will then yield levels in ppb (or ng/g) of Maduramicin by interpolation using the standard curve.

The concentrations of the samples are determined using the standard curve run with each test. Sample extracts showing a lower concentration of Maduramicin than standard 1 (0.25 ppb) should be reported as containing < 30 ppb of Maduramicin. Samples showing a higher concentration than standard 4 (2.5 ppb) must be diluted further with the provided sample diluent and re-analyzed. Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbances of the calibrators. Samples with lower absorbances than a calibrator will have concentrations of Maduramicin greater than the concentration of that calibrator. Samples which have higher absorbances than a calibrator will have concentrations of Maduramicin less than that calibrator.
Sensitivity

The limit of quantitation for Maduramicin (90% B/B0 calculated from the average of 30 calibration curves) is approximately 0.09 ng/mL. The middle of the test (50% B/B0 calculated from the average of 30 calibration curves) is approximately 0.74 ng/mL (average of 30 calibration curves). Determinations closer to the middle of the calibration curve give the most accurate results.

Specificity

Cross-reactivity of the Maduramicin Kit for related Ionophores:
Lasalocid, Monensin, Narasin, Salinomycin, Semduramicin < 0.1 %.

Reproducibility

Coefficients of variation (CVs) for standards: <10%; CVs for samples: <15%.

Precautions

The standard solutions in the test kit contain small amounts of Maduramicin. In addition, the substrate solution contains tetramethylbenzidine and the stop solution contains diluted sulfuric acid. Avoid contact of stopping solution with skin and mucous membranes. If these reagents come in contact with skin, wash with water.

Limitations

Although many organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test, due to the high variability of compounds that might be found in samples, test interferences caused by matrix effects can not be completely excluded. Samples must be extracted and diluted before testing in the ELISA. Mistakes in handling the test also can cause errors. Possible sources for such errors include: inadequate storage conditions of the test kit, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme temperatures (lower than 10°C or higher than 30°C) during the test performance.