Importance of Monensin Determination

The group of naturally occuring compounds known as “ionophores” (ion bearer) are carboxylic polyether antibiotics, which were initially developed for use in the prevention and treatment of coccidiosis in poultry. Ionophores form complexes with alkaline cations, creating lipophilic channels through hydrophobic lipid membranes. This facilitates the movement of metal ions across the membrane, interfering with the osmotic pressure of the cell. Ionophores such as Monensin, Lasalocid, Maduramicin, Narasin, Salinomycin and Semduramicin are active against Gram-positive bacteria, mycobacteria, some fungi and certain parasites and coccidia.

In addition to their use in the treatment and prevention of infection, Ionophores are also used at sub-therapeutic levels to improve feed efficiency in livestock. They are generally administered as feed additives. The withdrawal period for ionophores varies between 3-5 days. Although generally considered safe and effective at therapeutic doses in target animal species, accidental overdose, misuse, mixing errors, and accidental ingestion in non-target species can result in toxicity in a number of animals. Horses, certain avian species, dogs, and cats are especially sensitive to Ionophore toxicity. Effects of Ionophore toxicity include muscle degeneration, neuropathy, and cardiac toxicity and are often fatal.

To protect humans, regulatory agencies around the world have imposed regulatory limits regarding the amount of each Ionophore allowable in products for human consumption, such as poultry and edible tissue from other animals. In the US, the maximum residue permitted is 0.05 μg/g of edible cattle or goat tissues. The Acceptable Daily Intake (ADI) is 12.5 μg/kg of body weight per day.

The ABRAXIS® Monensin ELISA allows for the analysis of 43 samples in duplicate determination. Less than 1 mL of sample extract is required. The test can be performed in less than 2 hours.

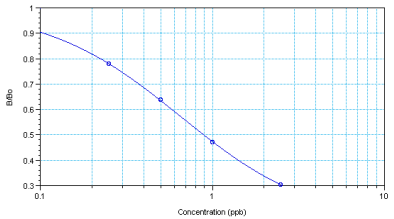
Performance Data

Test sensitivity: The limit of quantitation for Monensin is 0.176 ng/mL (mean of 20 blank determinations plus 4 standard deviations). The middle of the test (50% B/B_0) is 0.889 ng/mL (average of 30 calibration curves). Determinations closer to the middle of the calibration curve give the most accurate results.

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

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

Standard Curve:



For demonstration purposes only. Not for use in sample interpretation.

General Limited Warranty: Gold Standard Diagnostics warrants the products manufactured by the Company, against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product’s printed expiration date. **Gold Standard Diagnostics makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose**

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ABRAXIS® Monensin ELISA Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of Monensin in Feed and Contaminated Samples

Product No. 515785

1. General Description

The ABRAXIS® Monensin ELISA is an immunoassay for the quantitative and sensitive screening of Monensin. This test is suitable for the quantitative and/or qualitative screening of Monensin in animal feed and contaminated products (refer to Sample Preparation, Section D). For additional matrices, contact Gold Standard Diagnostics technical services for application bulletins and/or specific matrix validation guidelines. Samples requiring regulatory action should be confirmed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions

The standard solutions in the test kit contain small amounts of Monensin. 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 the skin, wash with water.

3. Storage and Stability

The ABRAXIS® Monensin ELISA should be stored in the refrigerator (2-8°C). The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the last day of the month as indicated by the expiration date on the box.

4. Test Principle

The test is a direct competitive ELISA based on the recognition of Monensin by specific antibodies. Monensin, when present in a sample, and a Monensin-HRP analogue compete for the binding sites of sheep anti-Monensin antibodies in solution. The Monensin antibodies are then bound by a second antibody (donkey anti-sheep) 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 Monensin 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.

5. Limitations of the ABRAXIS® Monensin ELISA, Possible Test Interference

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 cannot be completely excluded.

Samples must be extracted and diluted as instructed in the sample preparation section (Section D) or appropriate technical bulletin before testing in the ELISA.

Mistakes in handling the test also can cause errors. Possible sources for such errors can be: 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, exposure to direct or indirect sunlight during the substrate reaction, extreme temperatures (lower than 10°C or higher than 30°C) during the test performance.

The ABRAXIS® Monensin ELISA kit provides screening results. As with any analytical technique (GC, HPLC, etc.), samples requiring some regulatory action should be confirmed by an alternative method.

6. Working Instructions

A. Reagents and Materials Provided

1. Microtiter plate (12 X 8 strips) coated with a secondary antibody, in a resealable aluminum pouch
2. Monensin Standards (5): 0, 0.25, 0.5, 1.0, 2.5 ng/mL (ppb), 1 mL each
3. Antibody Solution (sheep anti-Monensin), 6 mL
4. Monensin-HRP Conjugate Solution, 6 mL
5. ABRAXIS® Wash Buffer (5X) Concentrate, 100 mL, must be diluted before use, see Test Preparation (Section C)
6. Sample Diluent, 2 bottles, 25 mL each
7. Substrate (Color) Solution (TMB), 16 mL
8. Stop Solution, 12 mL (handle with care)

B. Additional Materials (not delivered with the test kit)

1. Micro-pipettes with disposable plastic tips (50-200 µL)
2. Multi-channel pipette (10-300 µL), stepper pipette (10-300 µL), or electronic repeating pipette with disposable plastic tips (capable of delivering 50-300 µL))
3. Microtiter plate reader (wave length 450 nm)
4. Overhead tube rotator
5. Vortex mixer
6. Deionized or distilled water
7. Acetone, HPLC 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

C. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. The use of a multi-channel pipette, stepping pipette, or electronic repeating pipette is recommended for the addition of the antibody, enzyme conjugate, substrate, and stop solutions in order to equalize the incubation periods on the entire microtiter plate. 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 (2-8°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 ABRAXIS® 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 H_2SO_4.

D. Sample Preparation

Animal Feed

Animal feed, such as dog or cat food, which is in pressed pellet form, should be ground into a coarse powder before extraction. Moist samples should be homogenized into a paste-like consistency using a food processor or blender. Samples should be analyzed immediately after extraction.

1. Weigh 1.0 g of sample 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 30 minutes.
5. Centrifuge for 5 minutes at 3000 x g. Alternatively, a 1 mL aliquot can be transferred to a microcentrifuge tube and centrifuged for 5 minutes at ≥ 3000 x g for 5 minutes.
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 (Assay Procedure, step 1).

The Monensin 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.

E. Working Scheme

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; 0.5; 1.0; 2.5 ppb)

Samp1, Samp2, etc.: Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 0	Std 4										
B	Std 0	Std 4										
C	Std 1	Samp1										
D	Std 1	Samp1										
E	Std 2	Samp2										
F	Std 2	Samp 2										
G	Std 3	etc.										
H	Std 3	etc.										

F. Assay Procedure

1. **Add 50 µL of the standards or sample extracts** (Section D) 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, stepping, or electronic repeating pipette.
3. **Add 50 µL of antibody solution** to the individual wells successively using a multi-channel, stepping, or electronic repeating 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. After incubation, remove the covering, decant the contents of the wells into a sink, and blot the inverted plate on a stack of paper towels. **Wash the strips four times using the diluted wash buffer**. Use at least a volume of 250 µL of 1X wash buffer for each well and each washing step. Blot the inverted plate after each wash step on a stack of paper towels. After the last wash/blot, check the wells for any remaining buffer in the wells, and if necessary, remove by additional blotting.
6. **Add 150 µL of substrate (color) solution** to the individual wells successively using a multi-channel, stepping, or electronic repeating 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 30-60 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 using a multi-channel, stepping, or electronic repeating pipette.
8. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of stopping solution.

G. 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 $\%B/B_0$ 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 $\%B/B_0$ for each standard on the vertical linear (y) axis versus the corresponding Monensin concentration on the horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for samples will then yield levels in ppb or ng/mL of Monensin by interpolation using the standard curve. Results can also be obtained by using a spreadsheet macro available from Gold Standard Diagnostics upon request.

The concentrations of the samples are determined using the standard curve run with each test. Sample extracts showing a lower concentration of Monensin than standard 1 (0.25 ppb) should be reported as containing < 30 ppb of Monensin. Samples showing a higher concentration than standard 4 (2.5 ng/mL) 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 standards. Samples with lower absorbances than a standard will have concentrations of Monensin greater than the concentration of that standard. Samples which have higher absorbances than a standard will have concentrations of Monensin less than that standard.