Importance of Furazolidone Determination

Antibiotic residues in foods pose a serious threat to public health. The nitrofuran class of broad spectrum antibiotics (furaltadone, furazolidone, nitrofurantoin and nitrofurazone) are commonly used in food producing animals. Their potential for harmful effects on human health, specifically carcinogenicity, has led to bans on their use in food producing animals in many countries including the US, Canada, and the EU. These countries have also imposed bans on all imported foods containing nitrofuran residues. The monitoring of water sources and food products, such as meat, for antibiotic residues is necessary to ascertain that these compounds are not misused and do not present a danger to human and animal health.

The detection of nitrofurans themselves has proven challenging, as the drugs are rapidly metabolized after ingestion. The protein bound metabolites which are formed, however, persist in edible tissue for a considerable amount of time after treatment. AOZ (3-amino-2-oxazolidinone), the metabolite moiety derived from Furazolidone, is not degraded by common cooking techniques and can be released from tissue under mildly acidic conditions, making it ideal for monitoring and detection in edible tissue.

The Abraxis Furazolidone ELISA allows the determination of 41 samples in duplicate determination. Only a few grams or milliliters of sample is required. Hydrolysis and derivatization (overnight), and a subsequent solvent extraction step are necessary prior to assaying. The ELISA analysis can then be performed in less than 1 hour.

Performance Data

Test sensitivity: The limit of detection for Furazolidone, calculated as Xn +/- 3SD (n=20), in various matrices are as follows:

- Fish, shrimp, chicken, egg, milk, honey = 0.1 ppb
- Mixed feed = 0.3 ppb

Standard Curve: Determinations closer to the middle of the calibration range of the test yield the most accurate results.

These values are used for demonstration purposes only; do not use these values for your determinations.

Performance Data

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

Selectivity: This ELISA recognizes Furazolidone and not related compounds.

Cross-reactivities:
- Furazolidone (AOZ) = 100%
- Furaltadone (AMOZ) <0.01%
- Semicarbazide (SEM) <0.01%
- 1-aminoimidohydantoin (AHID) <0.01%

Samples: To eliminate matrix effects in fish, shrimp, and chicken, a sample clean-up is required. See Preparation of Samples, section H.

General Limited Warranty: Abraxis LLC 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. Abraxis makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.

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Furazolidone (AOZ) ELISA, Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of Furazolidone in Contaminated Samples

Product No. 515655

1. General Description

The Furazolidone ELISA is an immunoassay for the detection of the Furazolidone metabolite (AOZ). This test is suitable for the quantitative and/or qualitative detection of Furazolidone in contaminated samples. Samples requiring regulatory action should be confirmed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions

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

3. Storage and Stability

The Furazolidone ELISA Kit 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.

4. Test Principle

The Abraxis Furazolidone (AOZ) Plate Kit applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of Furazolidone. Calibrators (ready to use) and derivatized samples (please refer to reagent preparation section) are added, along with an AOZ-HRP enzyme conjugate, to wells in a microtiter plate that contain immobilized antibodies specific for AOZ. At this point, a competitive reaction occurs between the AOZ which may be in the sample and the enzyme labeled AOZ for the binding sites of the antibodies on the microtiter well. The reaction is allowed to continue for thirty (30) minutes. After a washing step, a substrate (Color Solution) is added. The presence of AOZ is detected by adding the "Color Solution," which contains the enzyme substrate (hydrogen peroxide) and the chromogen (3,3',5,5'-tetramethylbenzidine). The enzyme-labeled AOZ bound to the AOZ antibody catalyzes the conversion of the substrate/chromogen mixture to a colored product. After an incubation period, the reaction is stopped and stabilized by the addition of a diluted acid (Stopping Solution) and the color is evaluated using an ELISA plate reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run. Since the labeled AOZ (conjugate) was in competition with the unlabeled AOZ (sample) for the antibody sites, the color developed is inversely proportional to the concentration of AOZ in the sample.

5. Limitations of the Furazolidone ELISA, Possible Test Interference

Numerous organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in samples, test interferences caused by matrix effects can not be completely excluded. Mistakes in handling the test can also 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, extreme temperatures during the test performance (lower than 10°C or higher than 30°C).

The Abraxis Furazolidone ELISA Kit provides screening results. As with any analytical technique (GC, HPLC, etc.), samples requiring regulatory action should be confirmed by an alternative method.

Working Instructions

A. Materials Provided

1. Microtiter plate coated with antibodies against AOZ (12 X 8 strips).
2. Furazolidone Standards/Calibrators (7): 0, 0.0125, 0.025, 0.05, 0.1, 0.2, and 0.5 ppb, 1.0 mL each. Standards are ready to use (already derivatized).
3. Furazolidone-HRP Conjugate Solution, 12 mL.
4. Sample Treatment Buffer (10X) Concentrate, 50 mL (2 X 25 ml bottle). Use to dilute samples.
5. Wash Solution/Sample Diluent (10X) Concentrate, 50 mL.
6. Derivatization Reagent, 12 mL.
7. Color (Substrate) Solution (TMB), 12 mL.
8. Stop Solution, 12 mL.
B. Test Preparation
Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. We recommend using a multi-channel pipette or a stepping pipette for adding the conjugate, antibody, substrate and stop solutions in order to equalize the incubation periods of the solutions on the entire microtiter plate. Please use only the reagents and standards from one package kit 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 foil bag. The remaining strips are stored in the foil bag and zip-locked closed. Store the remaining kit in the refrigerator (4-8°C).
3. Dilute the Sample Treatment Buffer concentrate at a ratio of 1:10. If using both bottles (50 mL), add to 450 mL of distilled or deionized water.
4. Dilute the Wash Buffer/Sample Diluent concentrate at a ratio of 1:10. If using the entire bottle (50 mL), add to 450 mL of distilled or deionized water.
5. The Derivatization Reagent is prepared in DMSO (Dimethylsulfoxide). It will freeze when stored at 4-8°C. The reagent must be kept frozen at -20°C until use. All other reagents are stable at room temperature. Protect the strips from direct sunlight.

6. The standard solutions, conjugate, substrate and stop solutions are ready to use and do not require any further dilutions.
7. Dilute the Sample Treatment Buffer concentrate at a ratio of 1:10. If using both bottles (50 mL), add to 450 mL of distilled or deionized water.
8. The Derivatization Reagent is prepared in DMSO (Dimethylsulfoxide). It will freeze when stored at 4-8°C. The reagent must be kept frozen at -20°C until use. All other reagents are stable at room temperature. Protect the strips from direct sunlight.
9. The stop solution should be handled with care as it contains diluted H₂SO₄.

C. Assay Procedure
1. Weigh 1.0 g of homogenized fish or de-shelled shrimp (should have a paste-like consistency) or homogenized sample into a 50 mL centrifuge tube. Mix 1.0 g of sample with 4.0 mL of deionized water, 0.5 mL of 1M HCl, and 10 mL of hexane in a 50 mL centrifuge tube. Vortex for 1 minute.
2. Freeze at -20°C for 60 minutes, remove the upper liquid layer.
3. Melt the frozen layer, add 50 uL of Derivatization reagent and vortex for 1 minute. Follow step H a. 4 to 11 of the Fish/Shrimp/Egg Derivatization and Extraction procedure.

D. Evaluation
The evaluation of the ELISA can be performed using commercial ELISA evaluation programs 4-Parameter (preferred) or Logit/Log. For 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 Furazolidone concentration on the horizontal logarithmic (x) axis on graph paper. %B/B₀ for samples will then yield levels in ppb of Furazolidone by interpolation using the standard curve. Samples showing lower concentrations of Furazolidone compared to Standard 6 (0.0125 ng/mL) should be reported as containing < 0.05 ppb. Samples showing a higher concentration than Standard 6 (0.0125 ng/mL) must be diluted further to obtain accurate results.

E. Additional Materials (not included with the test kit)
1. Micro-pipettes with disposable plastic tips (10-200 and 200-1000 μL)
2. Multi-channel pipette (10-250 μL) or stepping pipette with plastic tips (10-250 μL)
3. Microtiter plate reader (wave length 450 nm)
4. Centrifuge, capable of spinning at 3,000 rpm
5. Vortex Mixer
6. 50 mL centrifuge tubes
7. 10 mL glass tubes or vials
8. Mixer (Stomacher, UltraTurrax)
9. Incubator (37°C)
10. Water bath (80-100°C)
11. Nitrogen (suggested but not essential)
12. Timer
13. Tape or Parafilm

F. Additional Reagents (not included with the test kit)
- Ethyl Acetate; n-Hexane; 1N HCl; 1 N NaOH; Distilled or Deionized water

G. 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.

H. Preparation of Samples
1. Weigh 1.0 g of homogenized fish or de-shelled shrimp (should have a paste-like consistency) or homogenized sample into a 50 mL centrifuge tube. Mix 1.0 g of sample with 4.0 mL of deionized water, 0.5 mL of 1M HCl, and 200 uL of Derivatization reagent. Vortex for 1 minute.
2. Follow steps H a. 3 to 11 of the Fish/Shrimp/Egg Derivatization and Extraction procedure.

NOTE:
An alternative derivatization (step 3 above) may be performed as follows: Incubate samples at 60°C for 3 hours. Proceed to step 4 above.

When using unknown samples of shrimp and fish with the incubation reduced from 16 hours at 37°C (section H, step 3) to 3 hours at 60°C, the recoveries were between 80-120% of those using the 16 hours at 37°C derivatization procedure.

When using known negative samples of shrimp and fish spiked and derivatized with the incubation reduced from 16 hours at 37°C (section H, step 3) to 3 hours at 60°C, the recoveries were between 70-110%.

For additional extraction procedures (mixed feed, chicken meat) please contact Abraxis LLC.