CALCULATE RESULTS

1. Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrator wells. Samples containing less color than a calibrator well have a concentration of AOZ greater than the concentration of the calibrator. Samples containing more color than a calibrator well have a concentration of AOZ less than the concentration of the calibrator.
2. Quantitative interpretation requires graphing the absorbances of the calibrators (X axis) versus the log of the calibrator concentration (Y axis) on semi-log graph paper. A straight line is drawn through the calibrator points and the sample absorbances are located on the line. The corresponding point on the Y axis is the concentration of the sample. Samples with absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as < 10 ppt or > 540 ppt, respectively.

TECHNICAL ASSISTANCE

For questions regarding this kit or for additional information about Beacon products, call (207) 571-4302.

SAFETY

To receive complete safety information on this product, contact Beacon Analytical Systems, Inc. and request Material Safety Data Sheets. Stop Solution is 1N hydrochloric acid. Handle with care.

General Limited Warranty

Beacon Analytical Systems, Inc. (“Beacon”) warrants the products manufactured by it against defects in materials and workmanship when used in accordance with the applicable instructions for a period not to extend beyond a product’s printed expiration date. BEACON MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The warranty provided herein and the data, specifications and descriptions of Beacon products appearing in published catalogues and product literature may not be altered except by express written agreement signed by an officer of Beacon. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and, if given, should not be relied upon.

In the event of a breach of the foregoing warranty, Beacon’s sole obligation shall be to repair or replace, at its option, any product or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies Beacon promptly of any such defect. The exclusive remedy provided herein shall not be deemed to have failed of its essential purpose so long as Beacon is willing and able to repair or replace any nonconforming Beacon product or part. Beacon shall not be liable for consequential, incidental, special or any other indirect damages resulting from economic loss or property damage sustained by a customer from the use of its products. However, in some states the purchaser may have rights under state law in addition to those provided by this warranty.

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Beacon Analytical Systems Inc.

AOZ Plate Kit

Cat.# 20-0177

Instructional Booklet

READ COMPLETELY BEFORE USE.

INTENDED USE

The Beacon AOZ Plate Kit is a competitive ELISA for the quantitative analysis of AOZ, a stable metabolite of nitrofuran antibiotic Furazolidone, in food samples.

USE PRINCIPLES

The Beacon AOZ plate kit is a competitive enzyme-labeled immunoassay. AOZ is extracted from samples by blending or shaking with extraction solvent. The extract is then derivatized with 2-Nitrobenzaldehyde to form NP-AOZ. AOZ-HRP enzyme conjugate is pipetted into the mixing wells followed by calibrators or sample extracts. The mixed solution is then transferred to antibody coated test wells. During the 30 minute incubation period, AOZ from the sample and AOZ-HRP enzyme conjugate compete for binding to the AOZ antibody. Following this 30 minute incubation, the contents of the well are removed and the wells are washed to remove any unbound AOZ or enzyme-labeled AOZ. A clear substrate is then added to the wells and any bound enzyme-AOZ conjugate causes the conversion to a blue color. Following a 30 minute incubation, the reaction is stopped and the amount of color in each well is read. The color of unknown samples is compared to the color of the calibrators and the AOZ concentration of the samples is derived.

MATERIALS PROVIDED

The kit in its original packaging can be used until the end of the month indicated on the box label when stored at 2 – 8ºC.

1 antibody coated plate containing 12 test strips of 8 wells each vacuum-packed in aluminized pouch with indicating desiccant
1 vial of Negative control (0 ppt NP-AOZ)
1 vial each of 10 ppt, 30 ppt, 90 ppt, 270 ppt, and 540 ppt NP-AOZ Calibrator
1 vial of AOZ-HRP Enzyme Conjugate
1 vial of Substrate
1 vial of Stop Solution (Caution! 1N HCl. Handle with care.)
1 Mixing wells
1 Sample derivative reagents
1 AOZ Sample buffer
1 Instruction booklet

MATERIALS REQUIRED BUT NOT PROVIDED:

- Laboratory quality distilled or deionized water.
- Glassware for sample extraction and extract collection
- Pipet with disposable tips capable of dispensing 100 µL.
- Multi-channel pipet; 8 channel capable of dispensing 100 and 150 µL.
- Paper towels or equivalent absorbent material.
- Microwell plate or strip reader with 450nm filter.
- Timer
- Vortex
- Wash bottle
- 1 M HCl
- 1 M NaOH
- 0.1 M K_2HPO_4
- Ethyl acetate
- n-hexane (or n-heptane)

PERFORMANCE CHARACTERISTICS

SPECIFICITY

The following table shows the % cross reactivity versus NP-AOZ.

Compound	% CR
NP-AOZ	100
NP-AMOZ	<1
NP-AHD	<1
NP-SCA	<1

PRECAUTIONS

- Each reagent is optimized for use in the Beacon AOZ Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon AOZ Plate Kits with different Lot numbers.
- Dilution or adulteration of reagents or samples not called for in the procedure may produce inaccurate results.
- Do not use reagents after expiration date.
- Reagents should be brought to room temperature, 20 – 28ºC (62 – 82ºF) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
- AOZ is an antibiotic and should be treated as such.
- The Stop Solution is 1N hydrochloric acid. Avoid contact with skin and mucous membranes. Immediately clean up any spills and wash area with copious amounts of water. If contact should occur, immediately flush with copious amounts of water.

SAMPLE PREPARATION

Meat sample (1:2 dilution)

1. Homogenize the meat using a stomacher, mixer, or ultra turrax.
2. Weigh out 1 g samples and add 4 mL of distilled water, 0.5 mL of 1M HCl, and 100 µL of sample derivative reagent. Mix vigorously for 3 minutes.
3. Incubate at 37°C overnight (approx. 16 h).
4. Add 5 mL of 0.1M K_2HPO_4, 0.4 mL of 1M NaOH, and 6mL of ethyl acetate. Mix vigorously for 1 minute.
5. Centrifuge for 5 minutes at approximately 3000g.
6. Transfer 3 mL of the ethyl acetate layer (upper layer) to a new tube and dry completely using a gentle nitrogen stream.
7. Dissolve the residue in 1 mL of n-hexane (or n-heptane) and mix properly with 0.5 mL of sample diluent.
8. Centrifuge for 5 minutes at approximately 3000g.
9. Discard the upper layer.
10. Transfer 100 µL and add 100 µL sample diluent.
11. Mix vigorously for 3 minutes and employ 100 µL per well in the assay.

ASSAY PROCEDURE

(Note: Running calibrators and samples in duplicate will improve assay precision and accuracy.)

1. Allow reagents and sample extracts to reach room temperature prior to running the test.
2. Place the appropriate number of test wells and mixing wells into a microwell holder. Be sure to re-seal unused wells in the zip-lock bag with desiccant.
3. Using a pipet with disposable tips, add **100 µL of calibrators or samples** to the appropriate mixing wells. Be sure to use a clean pipet tip for each.
4. Dispense **100 µL of AOZ-HRP conjugate** into each mixing well. Gently mix for at least 30 seconds.
5. Transfer **150 µL of the mixed solution** from the mixing wells into the appropriate test wells using a multi-channel pipet. Gently mix for at least 30 seconds.
6. Incubate the test wells for **30 minutes**.
7. Dump the contents of the wells into an appropriate waste container. Fill the wells to overflowing with clean laboratory grade water and dump. Repeat 3X for a total of four washes.
8. Following the last wash tap the inverted wells onto absorbent paper to remove the last of the wash solution.
9. Dispense **100 µL of Substrate** into each well.
10. Incubate the wells for **30 minutes** at 20—28°C.
11. Dispense **100 µL of Stop Solution** into each test well.
12. Read and record the absorbance of the wells at 450nm using a strip or plate reader.