Beacon Analytical Systems Inc.

Alternariol Plate Kit
Cat. # 20-0288

Instructional Brochure

READ COMPLETELY BEFORE USE

INTENDED USE

The Beacon Alternariol Plate Kit is a competitive ELISA for the quantitative analysis of alternariol in grains

USE PRINCIPLES

The Beacon Alternariol Plate Kit is a competitive enzyme-labeled immunoassay. Alternariol residues are extracted from a ground sample by shaking or blending with a 70% methanol/water solution. The extract is diluted, and tested in the immunoassay. Alternariol-HRP conjugate is pipetted into the test wells followed by sample extract or calibrators. An alternariol antibody is added into the test wells to initiate the reaction. During a 30 minute incubation period, alternariol from the sample and alternariol-HRP conjugate compete for binding to the alternariol antibody. Following this incubation, the wells are washed to remove any unbound alternariol or alternariol-HRP conjugate. After washing, a colorless substrate is added to the wells and any bound alternariol-HRP conjugate will convert the substrate to a blue color. Following a 30 minute incubation, the reaction is stopped and the amount of color in each well is measured. The color of the unknown sample is compared to the color of the calibrators and the alternariol concentration of the sample is derived. The color intensity is inversely proportional to the amount of alternariol present in the sample.

NOTE: Color is inversely proportionate to alternariol concentration
Darker color = Lower concentration
Lighter color = Higher concentration

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 to 8ºC.

1 Plate – containing 12 strips of 8 wells each, vacuum-packed in aluminized pouch with an indicating desiccant.
1 Vial Negative Control (0 ppb alternariol) – containing 2 mL.
4 Vials Alternariol Calibrators – containing 2 mL each, labeled as 0.45, 2.0, 5.0 and 7.5 ppb alternariol.
1 Bottle Alternariol HRP Enzyme Conjugate – containing 8 mL.
1 Bottle Alternariol Antibody Solution – containing 8 mL.
1 Bottle Substrate – containing 14 mL.
1 Bottle Stop Solution – containing 14 mL (Caution! Contains 1N HCl. Handle with care.)

MATERIALS REQUIRED BUT NOT PROVIDED

- Laboratory quality distilled or deionized water
- Pipette with disposable tips capable of dispensing 50 and 100 µL
- Multi-channel pipette with disposable tips; 8 channels capable of dispensing 50 and 100 µL
- Paper towels or equivalent absorbent material
- Timer
- Wash bottle
- Microtiter plate or strip reader with a 450 nm filter

*Additional materials may be required for sample preparation. See Sample Preparation Protocol.

SPECIFICITY

A number of toxic metabolites of the *Alternaria* fungi can be detected by this assay. The percent cross reactivity (% CR) of several of these compounds relative to alternariol is shown in the table below.

Compound		% CR
Alternariol	**(AOH)**	**100**
Alternariol monomethyl ether	**(AME)**	**0.25**
Tenuazonic acid	**(TeA)**	**<0.01**
Altenuene	**(ALT)**	**Not tested**
Tentoxin	**(TEN)**	**Not tested**

PRECAUTIONS

- Store all kit components at 2°C to 8°C (36°F to 46°F) when not in use.
- Each reagent is optimized for use in the Beacon Alternariol Plate Kit. Do not substitute reagents from any other manufacturer into the test kit.
- Do not combine reagents from other Beacon Abamectin Plate Kits with different lot numbers.
- Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
- Do not use reagents after expiration date. Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).
- Reagents should be brought to room temperature, 20 to 28ºC (62 to 82ºF) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
- Alternariol is a toxin. Handle using safety precautions.
- 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.
- Transfer of samples and reagents by pipette requires constant monitoring of technique. Pipetting errors are the major source of error in immunoassay methodology.
- Use approved methodologies to confirm positive results.
- Running calibrators and samples in duplicate will improve assay precision and accuracy.

EXTRACTION SOLUTION PREPARATION- 70% Methanol/Water

1. Carefully measure 30 mL of distilled or deionized water for each 100 mL being prepared and transfer to a clean glass container with a tight-fitting lid.
2. Carefully measure 70 mL of methanol for each 100 mL being prepared and add it to the container.
3. Cover and swirl to mix completely. Store the extract tightly sealed to minimize evaporation.

SAMPLE PREPARATION

Corn and other grain(s), Ketchup – Dilution Factor is 50X

Grind samples so that at least 95% of the ground material passes through a 20 mesh sieve and thoroughly mix prior to sub-sampling. Samples not immediately analyzed should be refrigerated or frozen.		
Extract Preparation Procedure		Example
1.	Weigh a representative sample and transfer to a clean blender jar with cap.	20 g
2.	Multiply the sample weight by 5 and add this volume of Extraction Solution (70% methanol/water) to the jar.	100 mL
3.	Shake by hand for 3 minutes, or blend for 1 minute in a high-speed blender.	5X Dilution
4.	Pipette ~1 ml of the top layer of sample extract into a disposable microcentrifuge tube.	
5.	Centrifuge in a microcentrifuge for 3 min. at 12,000 X g (RCF).	
6.	Dilute supernatant 1:10 (0.5 mL extract with 4.5 mL) in laboratory grade water and mix thoroughly.	10X Dilution
7.	Sample extract is ready for testing.	
	Total Dilution Factor =	**50 X**

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 into a micro well holder. Be sure to re-seal unused wells in the zip-lock bag with desiccant to limit exposure to moisture.
3. Dispense **50 µL of the HRP Enzyme Conjugate** into each well.
4. Dispense **50 µL of the Calibrators or Sample Extract** into the appropriate wells. Be sure to use a clean pipette tip for each well.
5. Dispense **50 µL of the Antibody Solution** into each well.
6. Shake the plate gently for 30 seconds using a back and forth motion.
7. Incubate the wells for **30 minutes** at room temperature.
8. Decant the contents of the wells into an appropriate waste container. Fill the wells to overflowing with laboratory grade water and then decant. Repeat four times for a total of five washes.
9. Following the last wash, tap the inverted wells onto absorbent paper to remove the last of the water.
10. Dispense **100 µL of the Substrate** into each well. Shake the plate gently for 30 seconds using a back and forth motion.
11. Incubate the wells for **30 minutes** at room temperature.
12. Dispense **100 µL of the Stop Solution** into each well. Shake the plate gently to mix the contents.
    WARNING!: Stop Solution is 1N HCl. Handle with care.
13. Measure and record the absorbance (Optical Density; OD) of the wells at 450 nm using a strip or plate reader.

CALCULATE RESULTS

Semi-Quantitative Interpretation- Semi-quantitative results can be derived by simple comparison of the sample absorbance to the absorbance of the calibrator wells. Samples with lower absorbance (less color) than a calibrator well, have a concentration of alternariol greater than the concentration of the calibrator. Samples with higher absorbance (more color) than a calibrator well have a concentration less than the concentration of the calibrator.

Quantitative Interpretation – Quantitative results require graphing the absorbance (OD) from the calibrator wells (Y axis) versus the calibrator concentration (X axis). This can be done using a plate reader with software which provides regression analysis using either a 4-Parameter or Semi Log curve fit.

Sample Results – Calculation of the concentration of target in the starting sample should be generated by multiplying the sample results from the standard curve by the **Total Dilution Factor** used during sample extract preparation. Samples with concentrations less than the first calibrator level are below the assay limit of detection (LOD$^{\lozenge}$). Samples with concentrations above the highest calibrator should be diluted 1:20 in water and rerun with a second standard curve. Please see example below.

SAMPLE CALCULATIONS

Actual values may vary; this data is for example purposes only.

Well Contents	Average OD ± SD*	RSD%	**Bo%	Sample conc. of Alternariol (X50)
0 ppb Calibrator	2.10 ± 0.05	2.4	100	0
0.45 ppb Calibrator	1.56 ± 0.04	2.6	74	22.5 ppb$^{\lozenge}$
2.0 ppb Calibrator	0.99 ± 0.04	3.9	47	100 ppb
5.0 ppb Calibrator	0.67 ± 0.02	2.2	32	250 ppb
7.5 ppb Calibrator	0.56 ± 0.02	2.7	27	375 ppb

* Standard deviation

**Bo% equals the average sample absorbance divided by the average 0 ppb Calibrator absorbance multiplied by 100.

TECHNICAL ASSISTANCE

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

SAFETY

To receive complete safety information on this product, contact Beacon Analytical Systems, Inc. and request 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.

Rev. 10/03/18

BEACON ANALYTICAL SYSTEMS, INC.
82 Industrial Park Road
Saco, ME 04072
Tel. (207) 571-4302
Fax (207) 602-6502
www.beaconkits.com