

## RESULTS INTERPRETATION

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 Ochratoxin greater than the concentration of the calibrator. Samples containing more color than a calibrator well have a concentration less than the concentration of the calibrator.
2. Quantitative interpretation requires graphing the absorbances of the calibrators (y axis) versus the log of the calibrator concentration (x 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 x 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 < 2 ppb or >25 ppb, respectively.

## SAMPLE CALCULATIONS

Well Contents	OD <sub>450</sub>	Average OD ± Std. Dev.	%RSD	%Bo*
0 ppb Calibrator	1.889 1.871	1.88 ± 0.013	0.7	100.0
2.0 ppb Calibrator	1.397 1.367	1.382 ± 0.021	1.5	73.5
4.0 ppb Calibrator	1.077 1.045	1.061 ± 0.023	2.1	56.4
10 ppb Calibrator	0.642 0.658	0.65 ± 0.011	1.7	34.6
25 ppb Calibrator	0.328 0.335	0.332 ± 0.005	1.5	17.6

This data is for example purposes only.

\* %Bo = (OD<sub>450</sub> / 0 ppb OD<sub>450</sub>) \* 100

## 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.



## Ochratoxin Plate Kit

Cat.# 20-0113

Instructional Booklet

READ COMPLETELY BEFORE USE.

### INTENDED USE

The Beacon Ochratoxin Plate Kit is a competitive ELISA for the quantitative analysis of Ochratoxin in corn, corn meal, and other grains.

BEACON ANALYTICAL SYSTEMS, INC.

82 Industrial Park Road

Saco, ME 04072

Tel. (207) 571-4302

Fax (207) 602-6502

[www.beaconkits.com](http://www.beaconkits.com)

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## ASSAY PRINCIPLES

The Beacon Ochratoxin Plate Kit is a competitive enzyme-labeled immunoassay. Ochratoxin is extracted from a ground sample by shaking with methanol/water. The extract is then filtered and the extract is tested in the immunoassay. Calibrators or sample extracts are pipetted into the mixing wells followed by Ochratoxin-HRP enzyme conjugate, this mixture is then added to the test wells. During the 10 minute incubation, Ochratoxin from the sample and Ochratoxin -HRP enzyme conjugate compete for binding to the anti-Ochratoxin antibody which is bound to the test well. Following this 10 minute incubation, the contents of the well are removed and the wells are washed to remove any unbound enzyme conjugate. A clear substrate is then added to the wells and any bound enzyme-toxin conjugate causes the conversion to a blue color. After a 10 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 Ochratoxin concentration of the samples is derived.

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## SPECIFICITY

The antibody utilized in the Beacon Ochratoxin Plate Kit is specific for ochratoxins. The calibrators are formulated with Ochratoxin A. Cross-reactivity with Ochratoxin B was determined to be 9.3% based on corresponding IC50.

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## PRECAUTIONS

1. Each reagent is optimized for use in the Beacon Ochratoxin Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Ochratoxin Plate Kits with different Lot numbers.
  2. Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
  3. Do not use reagents after expiration date.
  4. Reagents should be brought to room temperature, 20 – 28°C (62 – 82°F) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
  5. Ochratoxin is a toxic substance. Dispose of all liquids in a plastic container containing household bleach (minimum 10%). All labware should be soaked for at least 1 hour in a 30% solution of household bleach. Avoid contact of skin and mucous membranes with reagents and sample extracts by wearing gloves and protective apparel. If exposure of skin and mucous membranes to liquids should occur, immediately flush with water.
  6. 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.
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## REAGENTS AND 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 – 8°C.

- 1 Frame containing 12 test strips of 8 wells each vacuum-packed in aluminized pouch with indicating desiccant.
  - 5 vials each containing 2 mL of Ochratoxin calibrators in 70% methanol corresponding to 0, 2.0, 4, 10 and 25 ng/mL (ppb) of Ochratoxin. (Note: Because of the 1:5 dilution of the grain sample in the extraction step, the calibrators actually contain 1/5th of the stated value. No further correction back to the concentration in the original grain sample is required.)
  - 1 vial containing 12 mL of Ochratoxin-HRP Enzyme Conjugate.
  - 1 vial containing 14 mL of Substrate.
  - 1 vial containing 14 mL of Stop Solution. (Caution! 1N HCl. Handle with care.)
  - 1 bottle concentrated Wash Solution makes 500mL
  - 1 plate of red tabbed mixing wells
  - 1 Instructional Booklet
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## MATERIALS REQUIRED BUT NOT PROVIDED

- Laboratory quality distilled or deionized water.
  - Methanol, ACS grade
  - Graduated cylinder, 100 ml or larger.
  - Glassware for sample extraction and extract collection.
  - Filter, Whatman GF/A or equivalent
  - Pipet with disposable tips capable of dispensing 100 µL.
  - Multi-channel pipet; 8-channel capable of dispensing 100 µL.
  - Paper towels or equivalent absorbent material.
  - Microwell plate or strip reader with 450nm filter.
  - Timer
  - Wash bottle
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## EXTRACTION SOLUTION PREPARATION

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

1. Grind samples to pass a 20 mesh sieve and thoroughly mix prior to sub-sampling. Samples not being immediately analyzed should be stored refrigerated.
  2. Weigh 20 grams of ground sample and combine with 100 mL of 7:3, methanol: water (70% Methanol) in a clean container with tight fitting lid.
  3. Vigorously shake the container for 3 minutes.
  4. Allow sample to stand for 2-3 minutes to allow some settling of the slurry.
  5. Filter a minimum of 15 mL of the extract through Whatman GF/A filters and collect the extract into a clean container.
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## WASH SOLUTION PREPARATION

1. Transfer contents of Wash Concentrate bottle and 450mL lab grade water to a wash bottle. Swirl to mix.
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**TEST 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 strip holder. Be sure to re-seal unused coated test wells in the zip-lock bag with desiccant.
  3. Using a pipet with disposable tips, add **100 µL of calibrators and samples** to the mixing wells. Be sure to use a clean pipet tip for each.
  4. Dispense **100 µL of Enzyme Conjugate** into each mixing well.
  5. Using a multichannel pipet mix contents of mixing wells by slowly pipetting the liquid in and out of the tips 4 times. **Transfer 100 µL of the mixture to the test wells.**
  6. Incubate the test wells for **10 minutes**.
  7. Dump the contents of the wells into an appropriate waste container. Fill the wells to overflowing with diluted Wash Solution and dump wash. Repeat 4X for a total of five 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 **10 minutes**.
  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.
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