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 Zearalenone 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 < 0.02 ppm or >1 ppm, respectively.

SAMPLE CALCULATIONS

Well Contents	OD	Average OD ± SD*	%RSD	%Bo**	ZON conc. (ppb)
Negative Control	2.403 2.423	2.413 ± 0.014	0.6	100	N/A
20 ppb Calibrator	1.850 1.821	1.836 ± 0.021	1.1	76.1	N/A
50 ppb Calibrator	1.457 1.469	1.463 ± 0.008	0.6	60.6	N/A
250 ppb Calibrator	0.760 0.768	0.764 ± 0.006	0.8	31.7	N/A
1000 ppb Calibrator	0.383 0.372	0.377 ± 0.008	2.0	15.6	N/A
Sample	1.264 1.292	1.278 ± 0.020	1.5	53.0	75.5

Actual values may vary; this data is for example purposes only.
* standard deviation
** %Bo equals average sample absorbance divided by average negative control absorbance times 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.

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

Beacon Analytical Systems Inc.

Zearalenone Plate Kit

Cat.# 20-0019

Instructional Booklet

READ COMPLETELY BEFORE USE.

INTENDED USE

The Beacon Zearalenone Plate Kit is a competitive ELISA for the quantitative analysis of zearalenone in corn, corn meal, corn germ meal, corn gluten meal and corn/soy blend.

ASSAY PRINCIPLES

The Beacon Zearalenone kit is a competitive enzyme-labeled immunoassay. Zearalenone is extracted from a ground sample by shaking with methanol/water. The extract is filtered and diluted, the extract is then tested in the immunoassay. Zearalenone-HRP enzyme conjugate is pipetted into the mixing wells followed by calibrators or sample extracts. The sample-HRP mixture is then pipetted into the test wells to initiate the reaction. During the 10 minute incubation period, Zearalenone from the sample and Zearalenone-HRP enzyme conjugate compete for binding to Zearalenone 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-labeled toxin. A clear substrate is then added to the wells and any bound enzyme-toxin conjugate causes the conversion to a blue color. Following a 5 minute incubation, the reaction is stopped and amount of color in each well is read. The color of unknown samples is compared to the color of the calibrators and the Zearalenone concentration of the samples is derived.

SENSITIVITY

The Beacon Zearalenone Plate Kit is appropriate for the quantitative analysis of Zearalenone in grain and grain products in the range of 0.02 to 1.0 mg/kg(ppm). Samples containing less than 0.02 ppm should be reported as "< 0.02 ppm". Samples containing greater than 1.0 ppm should be reported as ">1.0ppm". Sample extracts containing greater than 1.0 ppm can be diluted with 70% methanol and re-analyzed to yield a quantitative result.

SPECIFICITY

The antibody utilized in the Beacon Zearalenone Plate Kit is specific for Zearalenone and closely related structures. The following table shows the relative reactivity for other forms:

Compound	% Cross-reactivity
Zearalenone	100%
a-zearalanol	30%
b-zearalanol	8%
a-zearalenol	Not tested
b-zearalenol	13%
zearalanone	81%

PRECAUTIONS

- Each reagent is optimized for use in the Beacon Zearalenone Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Zearalenone 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.
- Zearalenone is a very 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.
- 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.

REAGENTS AND MATERIALS PROVIDED

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

1 Plate containing 12 test strips of 8 wells each vacuum-packed in aluminized pouch with indicating desiccant.

5 vials each containing 2 mL of Zearalenone calibrators corresponding to 0, 0.02, 0.05, 0.25 and 1.0 µg/mL (ppm) of Zearalenone. (Note: Because of the 1:25 dilution of the grain sample in the extraction step, the calibrators actually contain 1/25th of the stated value. No further correction back to the concentration in the original grain sample is required.)

1 vial containing 12 mL of Zearalenone-HRP Enzyme Conjugate.

1 plate of red tabbed mixing wells.

1 vial containing 14 mL of Substrate.

1 vial containing 14 mL of Stop Solution. (Caution! 1N HCl. Handle with care.)

1 Instructional Booklet

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 - 200 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.

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.

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.
6. Dilute all sample extracts 1:5 with 70% methanol in a clean container**.**

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 into a microwell holder. Be sure to re-seal unused wells in the zip-lock bag with desiccant.
3. Place the same number of mixing wells as test wells into a microwell holder. Be sure to re-seal unused wells in the zip-lock bag.
4. Dispense **100 µL of standards or sample** into the appropriate mixing well.
5. Using a pipet with disposable tips, add **200 uL Enzyme conjugate to all** mixing wells.
6. Using a multichannel pipet, Mix the contents by gently pipetting the solution in and out 4 or 5 times before transferring **100 uL of the sample/HRP mixture** into the test wells.
7. Incubate the test wells for **10 minutes**.
8. Dump the contents of the wells into an appropriate waste container. Fill the wells to overflowing with tap water and dump. Repeat 4X for a total of five washes.
9. Following the last wash tap the inverted wells onto absorbent paper to remove the last of the wash solution.
10. Dispense **100 µL of Substrate** into each well.
11. Incubate the wells for **5 minutes**.
12. Dispense **100 µL of Stop Solution** into each test well.
13. Read and record the absorbance of the wells at 450nm using a strip or plate reader.