Quality Control (QC) Criteria

- The correlation coefficient (R^2) of the calibration curve, analyzed using a 4-parameter logistic regression, must be ≥ 0.99.
- The average absorbance of the zero calibrator replicates must be ≥ 1.0.
- The average absorbance of calibrator replicates must have a coefficient of variation (%CV) < 15%.
- The average absorbance of sample replicates must have a coefficient of variation (%CV) < 20%.

Result Interpretation

It is preferred for quantitative results to be determined using commercially available software for ELISA evaluation using a 4-parameter curve fit. Alternatively, a semi-log curve fit can be used if 4-parameter software is not available. A spreadsheet that will perform the curve fit and sample concentration calculations is available upon request. Please contact Beacon for further details.

To ensure the validity of the results, please adhere to the following:

- Ensure QC criteria are met.
- The concentration of ZON in a sample is determined by comparing the average sample absorbance to the standard curve. This value must then be multiplied by the dilution factor used.
- In the event that the average absorbance of the sample is lower than the highest calibrator, further dilute the sample extract in 16% methanol to fit into the standard curve and retest alongside the calibrators. Sample results must be multiplied by the total dilution factor used.

Technical Assistance

For questions regarding this kit or for additional information about Beacon products, contact us.

Beacon Analytical Systems, Inc.
82 Industrial Park Rd. Saco, ME 04046
Tel. +1-207-571-4302 | info@beaconkits.com | www.beaconkits.com

Safety

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

General Limited Warranty

Intended Use

The Beacon ZON 40 Tube Kit is an immunoassay for the detection of ZON in corn, corn meal, corn germ meal, corn gluten meal, and corn/soy blend samples. This product is intended for research use only.

Principles

ZON HRP Enzyme Conjugate is pipetted into the test tubes followed by the Calibrators and the Sample Extract(s). A soluble polyclonal ZON antibody solution is then added to the test tubes to initiate the reaction. During an incubation, ZON and ZON HRP Enzyme Conjugate compete for binding to the soluble ZON antibody which is in turn immobilized on the test tubes. Following the incubation, the tubes are washed to remove any unbound ZON and ZON HRP Enzyme Conjugate. After washing, a colorless substrate is added to the tubes and any bound enzyme conjugate will convert the substrate to a blue color. Following an incubation, the reaction is stopped with the addition of Stop Solution and the amount of color in each tube is measured. The color of the unknown sample is compared to the color of the calibrators and the ZON concentration of the sample is derived.

Reagents and Materials Provided

2 Units	Bags each containing 20 test tubes that are vacuum sealed in an aluminized pouch with a desiccant.
4 X 5 mL	Vials of ZON Calibrators (0, 10, 40, and 100 ppb). Note: The calibrators actually contain 1/10th of the stated value to account for the 1:10 dilution during sample preparation. No further correction is required to obtain the concentration of ZON in the sample.
1 X 24 mL	Bottle of ZON HRP Enzyme Conjugate.
1 X 24 mL	Bottle of ZON Antibody.
1 X 25 mL	Bottle of Substrate.
1 X 25 mL	Bottle of Stop Solution.

Reagents and Materials Required but Not Provided

- Pipette(s) with disposable tips capable of dispensing the required volume(s).
- Repeater pipette(s) with disposable tips capable of dispensing the required volume(s) (recommended if running more than five tubes at once).
- Laboratory quality distilled or deionized water.
- Reagents and materials for sample preparation.
- Personal protective equipment.
- Paper towels or equivalent absorbent material.
- Wash bottle (optional).
- Permanent Marker.
- Tube rack.
- Timer.
- Photometer capable of reading absorbance at 450 nm in 12 mm x 75 mm tubes.

Kit Handling Notes and Precautions

- Read the product brochure in its entirety prior to use.
- The kit, in its original packaging, can be used until the end of the month indicated on the box label.
- Do not use reagents after expiration date.
- Store all kit components at 2°C to 8°C (36°F to 46°F) when not in use.
- Reagents should be brought to room temperature, 20°C to 28°C (68°F to 82°F), prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
- Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).
- Running Calibrators and Samples in duplicate will improve assay precision and accuracy.
- Precise transfer of samples and reagents by using a calibrated pipette that is capable of dispensing the required volume is critical to obtain proper assay results.
- If running more than five tubes at once, the use of a repeater pipette is recommended when adding the Antibody, Substrate and Stop Solution.
- All procedural steps should be completed without interruption. Ensure all reagents, materials and equipment are ready at the appropriate time.
- Each reagent is optimized for use in the Beacon ZON 40 Tube Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon ZON 40 Tube Kits with different lot numbers.
- Do not reuse test tubes.
- Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
- Damage to or obstruction of the optical surface may cause unsatisfactory results.

Specificity

The antibody utilized in the Beacon ZON 40 Tube Kit is specific for ZON and closely related structures. The following table shows the relative reactivity for other forms.

Compound	% Cross-Reactivity
ZON	100
α-Zearalanol	30
β-Zearalanol	8
α-Zearalenol	Not Tested
β-Zearalenol	13
Zearalanone	81

Sample Extraction Solution Preparation (80% Methanol)

1. Measure 20 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. Measure 80 mL of methanol for each 100 mL being prepared and add to the container.
3. Cover and swirl to mix. Store tightly sealed to minimize evaporative loss.

Sample Preparation

<u>Corn, Corn Meal, Corn Germ Meal, Corn Gluten Meal, and Corn/Soy Blend:</u>
(Dilution Factor: 1)

The calibrators actually contain 1/10th of the stated value to account for the 1:10 dilution during sample preparation. No further correction is required to obtain the concentration of ZON in the sample.

1. Grind samples to pass through a 20 mesh sieve and thoroughly mix prior to sub-sampling. Samples not being immediately analyzed should be stored refrigerated.
2. Weigh 50 g of ground sample and add to a clean blender jar.
3. Weigh 5 g of NaCl and add to the jar.
4. Measure 100 mL of Sample Extraction Solution and add to the container.
5. Blend for 1 minute in a high-speed blender.
6. Filter a minimum of 10 mL of the extract into a clean container using a paper filter (a paper coffee filter is recommended).
7. Dilute the filtrate 1:5 in laboratory quality distilled or deionized water. Mix thoroughly.
8. Filter the diluted extract into a clean container using a glass fiber filter and use in the assay.

Assay Procedure

1. Allow kit components and the sample extract(s) to reach room temperature prior to running the test.
2. Place the appropriate number of test tubes into a tube rack. Label the tubes one inch from the top with the calibrator concentration or sample identification. Be sure to re-seal unused tubes in the zip-lock bag with the desiccant to limit exposure to moisture.
3. Dispense **500 µL of Enzyme Conjugate** into each tube.
4. Dispense **100 µL of Calibrators and Sample Extract(s)** into the appropriate tube. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
5. Dispense **500 µL of Antibody** into each tube.
6. Gently shake the tubes for 30 seconds using a back-and-forth motion and incubate for **10 minutes** at room temperature.
7. Decant the contents of the tubes into an appropriate waste container. Fill the tubes to overflowing with laboratory quality distilled or deionized water and then decant. Repeat this wash step three times for a total of four washes. Following the last wash, tap the inverted tubes onto absorbent paper to remove excess wash solution.
8. Dispense **500 µL of Substrate** into each tube.
9. Gently shake the tubes for 30 seconds using a back-and-forth motion and incubate for **10 minutes** at room temperature.
10. Dispense **500 µL of Stop Solution** into each tube in the same order of addition as the Substrate.
11. Gently shake the tubes for 30 seconds using a back-and-forth motion.
12. Carefully wipe the optical surface with a soft, lint-free wipe. Measure and record the absorbance (Optical Density; OD) of each tube at 450 nm using a tube reader within 10 minutes of stopping the assay. Be sure to blank the reader with laboratory quality distilled or deionized water prior to measuring.
13. Dispose of used test tubes in an appropriate waste container.