CALCULATE RESULTS

1. Semi-quantitative results can be derived by simple comparison of the sample absorbance's to the absorbance of the calibrator wells: Sample containing less color than a calibrator well have a concentration of Citrinin 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). A 4-parameter curve fit is recommended. If the plate reader software for this fit is unavailable, a spreadsheet that will perform the curve fit and sample calculations is available on our website, www.beaconkits.com or can be provided upon request. Samples with absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as <2 ppb or >200 ppb, respectively.

SAMPLE CALCULATIONS

Well Contents	OD		Mean OD	SD*	%RSD	%Bo**
0 ppb	1.654	1.601	**1.627**	0.038	2.3	**100%**
1 ppb	1.240	1.321	**1.281**	0.057	4.5	**79%**
10 ppb	0.761	0.805	**0.783**	0.031	3.9	**48%**
100 ppb	0.360	0.350	**0.355**	0.007	2.0	**22%**

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.

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Beacon Analytical Systems Inc.

Citrinin Plate Kit

Cat.# 20-0264

Instructional Booklet

READ COMPLETELY BEFORE USE.

INTENDED USE

The Citrinin Plate Kit is a competitive ELISA for the quantitative analysis of Citrinin for food safety purposes (example: red yeast rice and red yeast rice supplements).

USE PRINCIPLES

The Citrinin kit is a competitive enzyme-labeled immunoassay. Citrinin is extracted from a ground sample by shaking or blending with methanol/water. The extract is diluted then filtered and tested in the immunoassay. Citrinin-HRP enzyme conjugate is pipetted into the test wells followed by calibrators or sample extracts. Citrinin antibody is then pipetted into the test wells to initiate the reaction. During the 20 minute incubation period, Citrinin from the sample and Citrinin-HRP enzyme conjugate compete for binding to the Citrinin antibody which, in turn, binds to the test well. Following this 20 minute incubation, the contents of the well are removed and the wells are washed to remove any unbound toxin or 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 10 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 Citrinin concentration of the samples is derived.

MATERIALS PROVIDED IN THE BEACON CITRININ PLATE

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 yr from date of manufacture).

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

4 vials, each containing 2 mL of Citrinin calibrators corresponding to 0, 1, 10 and 100 µg/L (ppb) of Citrinin.

1 vial containing 8 mL of Citrinin-HRP Enzyme Conjugate.

1 vial containing 8 mL of Rabbit anti-Citrinin antibody.

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 paper (Fisher Scientific, P5) or equivalent.
- Pipette with disposable tips capable of dispensing 50 µL.
- Multi-channel pipet; 8 channel capable of dispensing 50 and 100 µL.
- Paper towels or equivalent absorbent material.
- Microwell plate or strip reader with 450nm filter.
- Centrifuge and Timer.
- 10 mM PBS, pH 7.2 [0.34 g of $NaH_2PO_4.H_2O$ (F.W. 137.99) + 1.08 g of Na_2HPO_4 (F.W. 141.96) + 8.5 g of NaCl, filling with 1 liter of laboratory grade water]

PRECAUTIONS

1. Each reagent is optimized for use in the Beacon Citrinin Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Citrinin 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 – 28ºC (62 – 82ºF) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
5. Citrinin is a very toxic substance. Dispose of all liquids in a plastic container containing household bleach (minimum 10%). All lab ware should be soaked for at least 1 hour in a 10% 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.
7. The intended user of this kit is a trained laboratory technician. Familiarity with ELISA is recommended. Please contact Beacon for technical support if you have any questions about the use of this kit.
8. The use of a multichannel pipette to dispense the generic reagents (Conjugate, Antibody, Substrate and Stop) is recommended when running 2 strips or more.

EXTRACTION SOLUTION PREPARATION

1. Carefully measure 90 mL of 10mM PBS for each 100 mL being prepared and transfer to a clean glass container with tight-fitting lid.
2. Carefully measure 10 mL of Methanol (MeOH) 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. Weigh 10 g of red yeast rice in a 50 mL centrifuge tube and add 20 mL of 10% MEOH / 10mM PBS.

   (For supplement samples, weigh 1 g of sample in a 20 mL scintillation vial and add 10 mL of 10% MEOH / 10mM PBS)
2. Vortex periodically for 30 min and filter with paper filter.
3. Transfer 1 mL into a micro centrifuge tube and centrifuge for 2 minutes at 10,000 rpm.
4. Dilute with 10% MEOH / 10mM PBS as needed.

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 microwell holder. Be sure to re-seal unused wells in the zip-lock bag with desiccant.
3. Using a pipette with disposable tips, add **50 µL of Enzyme Conjugate** into each test well. Add **50 µL of calibrators and samples** to the appropriate test wells. Be sure to use a clean pipet tip for each.
4. Dispense **50 µL of Antibody Solution** into each test well.
5. Shake the plate gently to mix contents, incubate the test wells for **20 minutes**.
6. Decant the contents of the wells into an appropriate waste container. Fill the wells to overflowing with Laboratory quality distilled or deionized water and dump. Dump the contents of the wells Repeat 4X for a total of five washes.
7. Following the last wash, tap the inverted wells onto absorbent paper to remove the last of the wash solution.
8. Dispense **100 µL of Substrate** into each well.
9. Shake the plate gently. Incubate the wells for **10 minutes**.
10. Dispense **100 µL of Stop Solution** into each test well. Shake the plate gently to mix.
11. Read and record the absorbance of the wells at 450nm using a strip or plate reader.