Zearalenone Plate Kit

PN 53018B

INTENDED USE
The Abraxis 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 Abraxis Zearalenone kit is a competitive enzyme-labeled immunoassay. Zearalenone is extracted from a ground sample by shaking with methanol/water. The extract is then filtered and diluted, the extract is then tested in the immunoassay. Zearalenone-HRP enzyme conjugate is pipetted into 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, in turn, binds 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.

SPECIFICITY
The antibody utilized in the Abraxis 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%

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 – 8ºC.

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

- 5 vials each containing 2 mL of Zearalenone calibrators corresponding to 0, 20, 50, 250,1000 ppb (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 25 mL of Zearalenone-HRP Enzyme Conjugate.
- 1 plate of red tabbed mixing wells.
- 1 vial containing 12 mL of Substrate.
- 1 vial containing 12 mL of Stop Solution. (Caution! 1N HCl. Handle with care.)
- Instructions

PRECAUTIONS

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

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.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Laboratory quality distilled or deionized water.

2. Methanol, ACS grade

3. Graduated cylinder, 100 ml or larger.

4. Glassware for sample extraction and extract collection.

5. Filter paper, Whatman GF/A or equivalent

6. Pipet with disposable tips capable of dispensing 50 µL.

7. Multi-channel pipet; 8 channel capable of dispensing 50 and 100 µL or Eppendorf Repeater pipette and tips for dispensing 50 and 100 µL.

8. Paper towels or equivalent absorbent material.

9. Microwell plate or strip reader with 450nm filter.

10. 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 70% MeOH/water 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 filter and collect the extract into a clean container.

6. Dilute all sample extracts 1:5 with 705 Methanol/water 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 and into a microwell holder. Be sure to re-seal unused wells in the zip-lock bag with dessicant.

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.

4. Using a pipet with disposable tips, add **100 µL of calibrators and samples** to the appropriate mixing wells. Be sure to use a clean pipet tip for each.

5. Using a pipette with disposable tips, dispense **200 µL of Enzyme Conjugate** into each test well.

6. Using a multichannel pipette. 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.

RESULTS INTERPRETATION

1. Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrator wells: Sample 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 (X axis) versus the log of the calibrator concentration (Y 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 Y 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.

TECHNICAL ASSISTANCE

For questions regarding this kit or for additional information about Abraxis products, call (215) 357-3911.

• ASSISTANCE

For ordering or technical assistance contact:

Abraxis LLC
54 Steamwhistle Drive
Warminster, Pennsylvania, 18974

Phone: (215) 357-3911 * Fax: (215) 357-5232
Email: info@abraxiskits.com
WEB: abraxiskits.com

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