

Fumonisin Plate Kit

PN 53014B

INTENDED USE

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

ASSAY PRINCIPLES

The Fumonisin kit is a competitive enzyme-labeled immunoassay. Fumonisin is extracted from a ground sample by shaking with methanol/water. The extract is then filtered and the extract is tested in the immunoassay. Fumonisin-HRP enzyme conjugate is pipetted into the test wells followed by calibrators or sample extracts. Fumonisin antibody is then pipetted into the test wells to initiate the reaction. During the 10 minute incubation period, fumonisin from the sample and fumonisin-HRP enzyme conjugate compete for binding to fumonisin 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 Fumonisin concentration of the samples is derived.

SPECIFICITY

The antibody utilized in the Abraxis Fumonisin Plate Kit is specific for fumonisins. The calibrators are formulated with fumonisin B₁. The following table shows the relative reactivity for other forms:

<u>Compound</u>	<u>Cross-Reactivity</u>
fumonisin B2	44%
fumonisin B3	104%

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.

- Plate containing 12 test strips of 8 wells each vacuum-packed in aluminized pouch with indicating desiccant.
- 5 vials each containing 2 mL of Fumonisin calibrators corresponding to 0, 0.3, 1.0, 3.0 and 6.0 $\mu\text{g/mL}$ (ppm) of Fumonisin. (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 8 mL of Fumonisin-HRP Enzyme Conjugate.
- 1 vial containing 8 mL of Rabbit anti-Fumonisin antibody.
- 1 vial containing 14 mL of Substrate.
- 1 vial containing 14 mL of Stop Solution. (Caution! 1N HCl. Handle with care.)
- Instructions

PRECAUTIONS

1. Each reagent is optimized for use in the Abraxis Fumonisin Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Abraxis Fumonisin 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. Fumonisin 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 , Whatman GF/A or equivalent
6. Pipet with disposable tips capable of dispensing 50 μ L.
7. Multi-channel pipet; 8 channel capable of dispensing 50 and 100 μ L.
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% 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.

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. Dispense **50 µL of Enzyme Conjugate** into each test well.
4. Using a pipet with disposable tips, add **50 µL of calibrators and samples** to the appropriate test wells. Be sure to use a clean pipet tip for each.
5. Dispense **50 µL of Antibody Solution** into each test well.
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 tap water and dump. 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 **5 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.

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 Fumonisin 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 < 0.3 ppm or >6 ppm, respectively.

TECHNICAL ASSISTANCE

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

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