



A Screening Test for Rapid Detection of Fumonisin in Grain Samples.

ABOUT FUMONISIN

Fumonisin is an environmental toxin produced mainly by the molds *Fusarium moniliforme*, *F. verticelloides*, *F. proliferatum* and other *Fusarium* species that grow on agricultural commodities in the field or during storage. These mycotoxins have been found worldwide, primarily in corn. More than 10 types of fumonisin have been isolated and characterized. Of these, fumonisin B1, B2 and B3 are the major fumonisins produced. The most prevalent of these mycotoxins in contaminated corn is fumonisin B1, which is believed to be the most toxic.^{1,2}

INTENDED USE

The Abraxis Fumonisin Test is designed solely for use in preliminary screening of grain samples. It is a competitive inhibition immunoassay for the qualitative detection of fumonisin in samples such as corn, corn meal and rice. The test produces a positive result in grain samples containing fumonisin in quantities of 5 ppm or higher. The Abraxis Fumonisin Test provides only a preliminary qualitative analytical test result. Other methods must be used to obtain a more confirmed analytical result. Professional judgment should be applied to any test results, particularly when preliminary positive results are used. HPLC or GCMS are recommended as methods of choice for confirmation of positive results obtained with the Abraxis Fumonisin Test.

INTRODUCTION

The Abraxis Fumonisin Test is a qualitative one-step competitive inhibition immunoassay for the detection of fumonisin. It detects the presence of fumonisin by utilizing highly specific reactions between antibodies and fumonisin in grain samples.

PRINCIPLE

The specifically labeled drug (drug conjugate) competes for antibody binding sites with toxins that may be present in the grain sample. The test device consists of a membrane strip to which a conjugate of the toxin of interest is attached. A colloidal gold labeled antibody is located at one end of the membrane. A control line, produced by a different antibody/antigen reaction, is also present on the membrane strip. The control line is not influenced by the presence or absence of mycotoxins in the grain sample, and therefore, it should be present in all reactions. In the absence of toxin in the grain sample, the colloidal gold labeled antibody complex moves with the grain sample by capillary action to contact the immobilized drug conjugate. An antibody-antigen reaction occurs forming a visible line in the 'test' area. **The formation of two visible lines indicates a negative test result. This means the test did not detect the toxin at or below the cut-off point established for the toxin.**

When fumonisin is present in the grain sample, it competes with the immobilized toxin conjugate in the test area for the antibody binding sites on the colloidal gold labeled antibody complex. If a sufficient amount of toxin analyte is present, it will fill all of the available binding sites, thus preventing attachment of the labeled antibody to the toxin conjugate. **The formation of one visible line indicates a positive result.**

Fumonisin Test Strip 5 ppm

MATERIALS PROVIDED

1. Fumonisin Test Strips
2. Package insert sheet

MATERIALS REQUIRED BUT NOT SUPPLIED:

1. 15 or 50 ml screw cap vial for extracting specimen
2. Phosphate Buffered Saline (PBS)
3. 1.7 ml microcentrifuge tubes
4. Pipets to deliver 500 ul
5. Timer

WARNINGS AND PRECAUTIONS

1. This test is for *screening* use by professionals only.
2. Prior to use, ensure that the product has not expired by verifying that the date of use is prior to the expiration date on the label.
3. The desiccant vial containing the test strips must remain completely closed except for opening to remove test strips. When re-closing, snap lid firmly.
4. Avoid cross-contamination of grain samples by using a new container for each specimen.

STORAGE

The Abraxis Rapid Test should be stored at room temperature (15° to 30°C) or refrigerated (2° to 8°C). The test strips and grain extract should be at room temperature before using.

SAMPLE COLLECTION AND EXTRACTION

1. Collect the sample according to GIPSA² recommended procedure.
2. Add 10 ml PBS to a suitable screw cap container. Weigh 1 gm grain sample and add to buffer in container, tighten cap and shake vigorously for 3 minutes to extract fumonisin from grain sample.
3. Allow sediment to settle for 5 minutes. It is important to allow the recommended settling time. Excess sediment in the extracts can interfere with the flow of liquid and this may affect test results.
4. Test line intensity may be lighter when extracts are not fresh. Same day testing is recommended.

ASSAY PROCEDURE

1. Be certain the test strip and grain extract have equilibrated to room temperature before conducting any testing. Temperature variation can affect test results.
2. Transfer 500 ul of grain extract to a micro centrifuge tube.
3. Insert test strip (with arrows down) into extract and allow test to develop.
4. Read results after 10 minutes. (Negative results may be visible within approx. 3 minutes.)

INTERPRETATION OF RESULTS

Read results after five minutes.

Figure 1: Positive Result - Fumonisin level greater than 5 ppm. Lower Fumonisin levels produce test line of increasing intensity.

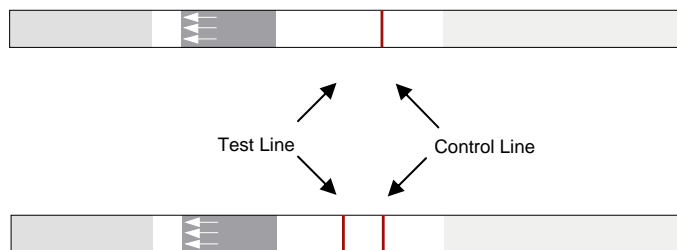


Figure 2: Negative Result - Fumonisin level less than 5 ppm

Test line is not visible in specimens with Fumonisin content greater than 5 ppm. Test line is visible with Fumonisin content less than 5 ppm.

TEST INTERPRETATION

Control Line	Test Line	Interpretation
No control line present	No test line present	Invalid result
Control line present	Distinct test line present	0 ppm
Control line present	No test line present	5 ppm and higher
Control line present	Light intensity test line present	Between 0 and 5 ppm

QUALITY CONTROL

It is good laboratory practice to use positive and negative controls to ensure proper test performance. Quality control materials are commercially available. It is recommended that each shipment of product be tested upon receipt.

LIMITATIONS OF PROCEDURE

The assay is designed for use with grain extracts only. The Abraxis Fumonisin Test provides only a preliminary qualitative test result. Use another more specific quantitative analytical method to obtain a confirmed analytical result.

Results obtained with the Abraxis Fumonisin Test cannot be considered conclusive evidence that fumonisin is present in quantities greater than the stated threshold. Specimens exhibiting positive results must be submitted to a qualified laboratory for analysis by GCMS or HPLC for definitive detection of fumonisin.

SENSITIVITY

The detection limit for fumonisin was established as 5 ppm as follows: Known concentrations of fumonisin were added to normal, toxin-free grain samples. Ten determinations were made at each serial dilution of the analyte. Sensitivity is defined as that concentration which produced positive responses in all 10 replicates.

REPRODUCIBILITY

Reproducibility studies were performed using extracts of grain samples that were previously assayed by HPLC.

PPM <u>Fumonisin</u>	No. <u>samples</u>	<u>Results</u>	<u>Precision</u>
0	30	Negative	100%
5.0	30	Positive	100%

BIBLIOGRAPHY

1. Thiel, P.G., Marasas W.F.O., Sydenham, E.W., Shephard, G.S. and Gelderblom, W.C.A. 1992. The implications of naturally occurring fumonisins in corn for human and animal health. *Mycopathologia* 117:3-9
2. Musser, S.M. and Plattner, R.D. 1997. Fumonisinb compositions in cultures of *Fusarium moniliforme*, *Fusarium proliferatum*, and *Furarium nygami*. *Journal of Agricultural and Food Chemistry* 45:1169-1173.