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See external label	2°C-8°C	96 tests	REF 5123-8

Enzyme Immunoassay for the Quantitative Determination of Fumonisin in Maize and Grain Products

Fumonisin

Cat #5123-8

Sensitivity	0.5 ng/mL
Recovery (spiked samples)	80%
Incubation Time	80 min

GENERAL INFORMATION

Fumonisin B1 and B2 are mycotoxins, which are produced by Fusarium moniliforme, F. solani and other types of fusarium. These toxins show an extreme stability against high temperatures (up to 100℃), and they can remain active in contaminated food for years. Fumonisin can be found in maize, oats and other types of grain. Worldwide a contamination in maize of 60% has been detected. When ingested by animals, fumonisin leads to neurotoxicity, hepatotoxicity and lung edema, mainly in horses and pigs. Therapeutic measures are the change of the grain given to the animals, or the administration of diuretic drugs. In human patients hints for the appearance of esophagus cancer could be associated with the exposition to fumonisin B1. Values assessed for the acute toxicity are 8 mg per kg weight and for the chronic situation 25 mg/kg in feed stuff. These are relatively high concentrations, but according to the Good Manufacturing Practice, the content of fumonisin B1 in food intended for humans should not exceed 1 mg/kg. The usual methods for detection of fumonisin in maize are liquid and thin-layer chromatography. The ELISA kit is very sensitive and allows the detection of trace amounts below 25 µg per kg.

PRINCIPLE OF THE TEST

The **Diagnostic Automation, Inc. Fumonisin** quantitative test is based on the principle of the enzyme linked immunosorbent assay. A microtiter plate is coated with antibodies raised against mouse immunglobulins. The standards and samples respectively are pipetted together with a fumonisin-peroxidase conjugate and a mouse-anti-fumonisin antibody into the appropriate wells. The conjugate competes with the fumonisin of samples/standards for the limited number of antibody sites. Simultaneously the anti-fumonisin antibody is bound to the anti-mouse antibody coated on the microtiter plate. After one hour incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A substrate solution is added and incubated for 20 minutes, resulting in the development of a blue colour. The colour development is inhibited by the addition of a stop solution, and the colour turns yellow. The yellow colour is measured photometrically at 450 nm. The concentration of fumonisin is indirectly proportional to the colour intensity of the test sample.

PRECAUTIONS

Full compliance of the following good laboratory practices (GLP) will determine the reliability of the results:

1. Prior to beginning the assay procedure, bring all reagents to room temperature (20-25℃).
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
4. Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
5. Use a separate disposable tip for each specimen to prevent cross-contamination.
6. All specimens and standards should be run at the same time, so that all conditions of testing are the same.
7. Do not mix components from different batches.
8. Do not use reagents after expiration date.
9. Check both precision and accuracy of the laboratory equipment used during the procedure (micro pipets, ELISA reader etc.).

HEALTH AND SAFETY INSTRUCTIONS

1. Do not smoke or eat or drink or pipet by mouth in the laboratory.
2. Wear disposable gloves whenever handling patient specimens.
3. Avoid contact of substrate and stop solution with skin and mucosa (possible irritation, burn or toxicity hazard). In case of contact, rinse the affected zone with plenty of water.
4. Handling and disposal of chemical products must be done according to good laboratory practices (GLP).

REAGENTS

The kit contains reagents for 96 determinations. They have to be stored at 2-8℃. Expiry data are found on the labels of the bottles and the outer package.

1. Microtiter plate consisting of 12 strips with 8 breakable wells each, coated with anti-mouse immunglobulin.
2. Fumonisin Standards (0; 0.5; 2; 5; 10; 25 ng/mL): 6 vials with 1.0 mL each, dyed red, ready-to-use.
3. Conjugate (Fumonisin-Peroxidase): 6 mL, dyed red, ready-to-use.
4. Anti-Fumonisin Antibody, 6 mL, dyed blue, ready-to-use.
5. Substrate Solution (TMB): 15 mL; ready-to-use.

6. Stop Solution (0.5 M H_2SO_4): 15 mL; ready-to-use.
7. Sample Diluent (PBS): 2 x 60 mL; dyed red, ready-to-use.
8. Washing Solution (PBS + Tween 20): 60 mL as 10x concentrate, dyed blue. Dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37℃ for 15 minutes.
9. Two plastic foils to cover the strips during the incubation.
10. Plastic bag to store unused microtiter strips.
11. Instruction Manual.

ADDITIONAL INSTRUMENTATION AND REAGENTS

(not provided)

Instrumentation

- 50, 100 and 1000 µL-micropipets
- ELISA reader (450 nm)
- Grinder
- Centrifuge

Reagents

- Methanol (80%)

SAMPLE PREPARATION

- Grind approximately 50-100 g of maize or grain sample to a fine powder.
- Extract 3 g ground sample with 9 mL 80% methanol in distilled water on a shaker for at least 15 minutes.
- Clear the sample by centrifugation (10 min, 2000 g) or filtration.
- Dilute cleared sample 1:15 in sample diluent (e.g. 100 µL cleared sample + 1.4 mL sample diluent).

PROCEDURE

1. Prepare samples as described above.
2. Pipet 100 µL standards or prepared samples in duplicate into the appropriate wells of the microtiter plate. Immediately add 50 µL fumonisin-peroxidase conjugate and 50 µL anti-fumonisin antibody into each well.
3. Cover the microtiter plate with a plastic foil and incubate for 60 minutes at room temperature.
4. Wash the plate three times as follows: Discard the contents of the wells (dump or aspirate). Pipet 300 µL of diluted washing solution into each well. After the third repetition empty the wells again and remove residual liquid by striking the plate against a paper towel. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbencies.
5. Pipet 100 µL of substrate solution into each well.
6. Allow the reaction to develop in the dark (e.g. cupboard or drawer; the chromogen is light-sensitive) for 20 minutes at room temperature.
7. Stop enzyme reaction by adding 100 µL of stop solution (0.5 M H_2SO_4) into each well. The blue colour will turn yellow upon addition.
8. After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The colour is stable for 30 minutes.

CALCULATION OF RESULTS

1. Calculate the average optical density (OD 450 nm) for each set of reference standards or samples.
2. Construct a standard curve by plotting the mean optical density obtained for each reference standard against its concentration in ng/mL on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis.
3. Using the mean optical density value for each sample, determine the corresponding concentration of fumonisin in ng/mL from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. The diluted samples must be further converted by the appropriate dilution factor (45 for the above described extraction). The factor is dependent on the sample preparation procedure employed.

TYPICAL STANDARD VALUES

The following table contains an example for a typical standard curve. The binding is calculated as percent of the absorption of the 0 ng/mL standard. These values are only an example and should not be used instead of the standard curve which has to be measured in every new test.

Fumonisin (ng/mL)	(% binding of 0 ng/mL)
0	100
0.5	87
2	62
5	38
10	23
25	11

PERFORMANCE

Sensitivity

The sensitivity of the **Diagnostic Automation, Inc. Fumonisin test** is 0.5 ng/mL (based on the standard curve).

Recovery

The recovery of spiked samples was determined to 80% for maize products.

Intra-assay Precision

The intra-assay variation of the fumonisin test was determined to 3%.

REFERENCES

1. Bauer J & Binder S (1993) Fumonisin in Futtermitteln: Vorkommen und Bedeutung einer neuen Gruppe von Fusarientoxinen. Tierärztliche Umschau 48, 718-727
2. Colvin BM, Cooley AJ & Beaver RW (1993) Fumonisin toxicosis in swine: clinical and pathologic findings. J Vet Diagn Invest 5, 232-241
3. Colvin BM & Harrison LR (1992) Fumonisin-induced pulmonary edema and hydrothorax in swine. Mycopathologia 117, 79-82
4. Guzman RE, Casteel SW, Rottinghaus GE & Turk JR (1997) Chronic consumption of fumonisins derived from Fusarium moniliforme culture material: clinical and pathologic effects in swine. J Vet

Diagn Invest 9, 216-218
5. Haschek WA, Gumprecht LA, Smith G, Tumbleson ME & Constable PD (2001) Fumonisin toxicosis in swine: An overwiew of porcine pulmonary edema and current perspectives. Env Health Perspect Suppl 109, 251-257
6. Osweiler GD, Ross PF, Wilson TM, Nelson PE, Witte ST, Carson TL, Rice LG & Nelson HA (1992) Characterization of an epizootic of pulmonary edema in swine associated with fumonisin in corn screenings. J Vet Diagn Invest 4, 53-59
7. Pace L, Rottinhaus GE, Shelby R, Misfeldt M & Ross PF (1995) Effects of feeding fumonisin B_1 in lactating sows and their suckling pigs. Am J Vet Res 56, 1253-1258
8. Ross PF, Rice LG, Plattner RD, Osweiler GD, Wilson TM, Owens DL, Nelson HA & Richard JL (1991) Concentrations of fumonisin B_1 in feeds associated with animal health problems. Myco pathologia 114, 129-135
9. Rottinghaus GE, Coatney CE & Minor HC (1992) A rapid, sensitive thin layer chromatography pro cedure for the detection of fumonisin B_1 and B_2. J Vet Diagn Invest 4, 326-329
10. Straw BE, Dewey CE & Wilson MR (1999) Differential diagnosis of swine diseases. In: Diseases of Swine - 8th Edition (BE Straw, S D'Allaire, WL Mengeling & DJ Taylor ed.), Iowa State University Press, Ames, pp 41-88

Date Adopted	Reference No.
2010-01-21	DA-Fumonisin-2010

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ISO 13485-2003

Revision Date: 12-6-2013