Test Instruction

Aflatoxin M_1 RAPID

96 Tests

Enzyme Immunoassay
for the Rapid Quantitative
Determination of
Aflatoxin M_1 in in Milk and Milk
Products

Cat.-No.: AM1-E03

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Gold Standard Diagnostics Kassel GmbH

Otto-Hahn-Str. 16
D-34123 Kassel
Germany

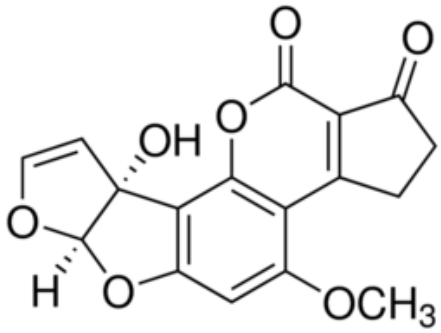
Phone: +49 (0) 561 491742-0
Fax: +49 (0) 561 491742-20

info.kassel@eu.goldstandarddiagnostics.com

www.kassel.goldstandarddiagnostics.com

Sensitivity	0.03 ppb
Recovery (spiked samples)	89-92%
Incubation Time	20 min

General Information



Aflatoxins belong to the class of mycotoxins. Chemically they are defined as difuranocyclopentanocumarines or difuranopentanolidocumarines, i.e. aflatoxins contain a dihydrofuran or a tetrahydrofuran ring, to which a substituted cumarin system is condensed. Out of about 20 known aflatoxins, the moulds *Aspergillus flavus* and *A. parasiticus* produce exclusively aflatoxin B_1, B_2, G_1 and G_2, and all the other aflatoxins are derivates of these four. The derivates are developed either by metabolism in humans, animals and microorganisms or by environmental reactions.
Aflatoxin M_1 was the first metabolite of aflatoxin B_1, which could unequivocally be detected by Allcroft and Carnaghan in the milk of cows in 1963. Out of this reason this first derivative was called aflatoxin M_1 (= milk). As further investigations showed, also other mammalians excrete aflatoxin M_1 in milk, feces and urine. Contaminations of milk and milk products can be hazardous for human beings, because M_1 is similar to aflatoxin B_1 regarding its hepatotoxicity. M_1 is only less carcinogenic.

In the USA, China and India the limits for afaltoxin M_1 in milk are set to the level of 0.5 ppb. Thus, a monitoring of milk and milk products with respect to the concentration of aflatoxin M_1 is obligatory.

Principle of the Test

The **Gold Standard Diagnostics Aflatoxin M_1 RAPID** quantitative test is based on the principle of the enzyme-linked immunosorbent assay. An antibody binding protein is coated on the surface of a microtiter plate. Aflatoxin M_1 containing samples or standards, an aflatoxin M_1-peroxidase conjugate and an antibody directed against aflatoxin M_1 are given into the wells of the microtiter plate. The conjugate competes with the aflatoxin M_1 of samples/standards for the limited number of antibody sites. Simultaneously the anti-aflatoxin M_1 antibody is bound to the antibody-binding protein coated on the microtiter plate. After 10 minutes incubation at room temperature the wells are washed with diluted washing solution to remove unbound material. A substrate solution is added and incubated for 10 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 aflatoxin M_1 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°C).
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 (micropipets, ELISA reader etc.).

Health and safety instructions

1. Do not smoke or eat or drink or pipet by mouth in the laboratory.
2. 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.
3. 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°C. 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 antibody-binding protein.
2. Aflatoxin M_1 Standards (0; 0.125; 0.3; 0.6; 1.2; 2.4 ppb): 6 vials with 1 mL each, in methanol, dyed red, ready-to-use.

   Since milk is directly applied to the test with no additional dilution, no further calculation after analysis is necessary.
3. Anti-aflatoxin M_1 Antibody (rabbit): 6 mL, dyed blue, ready-to-use.
4. Conjugate (aflatoxin-Peroxidase): 6 mL, dyed red, 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. Washing Solution (PBS + Tween 20): 60 mL as 10x concentrate. Dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.
8. Plastic bag to store unused microtiter strips.
9. Instruction Manual.

Additional Instrumentation and Reagents (not provided)

Instrumentation

- 50 and 100 µL- micropipets
- ELISA reader (450 nm)
- Centrifuge
- Ultra-Turrax, mixer, vortex

Reagents

- Double distilled water

Sample Preparation

Milk

- 5 mL of a fresh milk sample (full-cream milk or skim milk) are pipetted into a test tube and incubated for 30 minutes at 4°C. Alternatively this step can be skipped and a centrifuge cooled down to 4°C has to be used in the next step.
- Centrifuge at a minimum of 3000 g for at least 10 minutes.
- Discard the upper fat layer and apply the aqeous phase to the ELISA.

Milk Powder

- 10 g milk powder is dissolved in 100 mL of 50°C hot double-distilled water.
- The solution is homogenized by a magnetic stirrer.
- The homogenized solution is treated according to the above procedure for milk.

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.

3. Add 50 µL of aflatoxin M_1-peroxidase conjugate into each well.

4. Add 50 µL of the anti-aflatoxin M_1 antibody into each well.

5. Incubate for 10 minutes at room temperature.

6. 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.

7. Pipet 100 µL of substrate solution into each well.

8. Allow the reaction to develop in the dark (e.g. cupboard or drawer; the chromogen is light-sensitive) for 10 minutes at room temperature.

9. 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.

10. 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

The ready-to-use standards are prepared for a direct determination of milk samples. Additional dilution due to high sample concentration has to be accounted for.

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 ppb on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis. Alternatively the evaluation can be carried out by software. In this case the 4-parameter method should be preferred.

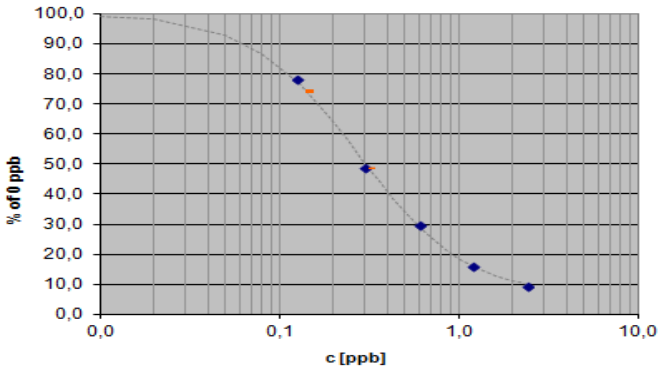
3. Using the mean optical density (OD) value for each sample, determine the corresponding concentration of aflatoxin M_1 in ppb from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.

4. Due to a deviating sample preparation process the results for milk powder samples additionally have to be multiplied with 10 in order to get the real concentration of the initial dry sample.

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 ppb standard. These values are only an example and should not be used instead of the standard curve which has to be measured in each new test.

Aflatoxin M_1 (ppb)	(% binding of 0 ppb)
0	100
0.125	78
0.3	49
0.6	30
1.2	16
2.4	9



Performance

Sensitivity

The limit of detection (LOD) of the **Gold Standard Diagnostics Aflatoxin M1 Rapid test** is 0.03 ppb for the standard curve.

The limit of quantification (LOQ) of the **Gold Standard Diagnostics Aflatoxin M1 Rapid test** is 0.11 ppb for the standard curve.

Validation experiments with common matrices resulted in the following LODs and LOQs [ppb].

Matrix	LOD	LOQ
Milk	0.03	0.12
Milk powder (reconstituted)	0.04	0.12

Recovery

Milk	92%
Milk Powder	89%

Linearity

The serial dilution of spiked samples (milk, milk powder) resulted in a dilution linearity of 93-106%.

Precision

Intra-assay Precision	3%
Inter-assay Precision	9%

References

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