RIDASCREEN® Aflatoxin M1

REF R1121

Enzymimmunoassay zur quantitativen Bestimmung von Aflatoxin M1

Enzyme immunoassay for the quantitative determination of aflatoxin M1

In vitro Test

Lagerung bei 2 - 8 °C
Storage at 2 - 8 °C

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RIDASCREEN® Aflatoxin M1

Brief information

RIDASCREEN® Aflatoxin M1 (R1121) is a competitive enzyme immunoassay for the quantitative analysis of aflatoxin M1 in milk and milk powder.

All reagents required for the enzyme immunoassay, including standards, are contained in the test kit. The test kit is sufficient for max. 96 determinations (including standards). A microtiter plate spectrophotometer is required for quantification.

Sample preparation:	milk……degreasing milk powder……dissolving and degreasing
Time requirement:	sample preparation (for 10 samples) milk …….... approx. 20 min milk powder ……. approx. 1 h test implementation (incubation time)…… 1 h 15 min
Limit of detection: (corresponding to the standard substance)	milk ……..…5 ng/l (ppt) milk powder ……5 ng/l (referring to dissolved milk) milk powder (referring to g-weight)……..50 ng/kg
Recovery rate: (corresponding to the standard substance)	in spiked samples milk ……..……..... 100 % milk powder……..... 104 %
Specificity:	aflatoxin M1…….... 100 % aflatoxin M2…….. < 10 %

Further information is contained in the validation report.

The specificity of the RIDASCREEN® Aflatoxin M1 test was determined by analyzing the cross reactivities to corresponding substances in buffer system. In samples, the specificity may deviate from those determined in the buffer system due to matrix effects. Prior to the analysis of cross-reactive substances, the user has to determine the Limit of Detection and the Recovery for the substance in the

respective sample matrix. The test cannot discriminate between analytes and cross-reactive substances.

In order to increase the quality of assessment when performing ELISA procedures, we refer additionally to our Good ELISA Practice manual. It lists minimum standards concerning the framework conditions when using test kits of R-Biopharm AG and performing ELISA analyses with them. The manual can be retrieved, printed and downloaded from www.r-biopharm.com/products/food-feed-analysis.

Related products

RIDASCREEN® FAST Aflatoxin M1 (R5812)
Trilogy® Liquid Standard Aflatoxin M1 (TSL-143-2)
Trilogy® Dried Standard Aflatoxin M1 (TS-130-2)

1. Intended use

RIDASCREEN® Aflatoxin M1 (R1121) is a competitive enzyme immunoassay for the quantitative analysis of aflatoxin M1 in milk and milk powder.

2. General information

Aflatoxins are carcinogenic, highly toxic metabolites of the mold fungus varieties *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin M1 is produced as a metabolite of aflatoxin B1. It is secreted with the milk after feeding of aflatoxin B1 containing feed to lactating cows. As aflatoxin M1 is relatively stable towards the pasteurizing process, not only a comprehensive routine check of the raw materials to be processed is required, but also of the final products.

Since the first of January 1999 EU-wide uniform residue limits for aflatoxins exist. For aflatoxin M1 in milk the limit has been fixed at 0.05 µg/l (50 ppt).

3. Test principle

The basis of the test is the antigen-antibody reaction. The wells in the microtiter strips are coated with capture antibodies directed against anti-aflatoxin M1 antibodies. Anti-aflatoxin M1 antibodies are added which are bound by the immobilized capture antibodies. After incubation and washing step, standards or samples are added. After a further incubation with a subsequent washing step, the aflatoxin M1 enzyme conjugate is added. Free and enzyme conjugated aflatoxin M1 compete for the antibody binding sites (competitive enzyme immunoassay). Any unbound enzyme conjugate is then removed in a washing step. The

substrate/chromogen is added to the wells and incubated. Bound enzyme conjugate converts the chromogen into a blue product.
The addition of the stop solution leads to a color change from blue to yellow. The measurement is made photometrically at 450 nm and the absorption is inversely proportional to the aflatoxin M1 concentration in the sample.

4. Reagents provided

Each kit contains sufficient materials for max. 96 measurements (including standard analyses). Each test kit contains:

Component	Cap color	Format		Volume
Microtiter plate K	-	Ready to use		96 wells
Sample buffer	Transparent	Ready to use		60 ml
Standard 1	White	Ready to use	0 ng/l	1.3 ml
Standard 2	White	Ready to use	5 ng/l	1.3 ml
Standard 3	White	Ready to use	10 ng/l	1.3 ml
Standard 4	White	Ready to use	20 ng/l	1.3 ml
Standard 5	White	Ready to use	40 ng/l	1.3 ml
Standard 6	White	Ready to use	80 ng/l	1.3 ml
Wash buffer salt Tween		Dissolve the salt		
Conjugate	Red	Ready to use		11 ml
Antibody	Black	Ready to use		11 ml
Substrate/Chromogen Red Chromogen Pro	Brown	Ready to use		10 ml
Stop solution	Yellow	Ready to use		14 ml

5. Reagents required but not provided

5.1 Equipment

- Balance
- Graduated pipettes
- Stirrer / shaker (for milk powder samples)
- Centrifuge + centrifugal vials
- Pasteur pipettes
- Micropipettes for 100 µl and 250 µl
- Microtiter plate spectrophotometer (450 nm)

5.2 Reagents

– Distilled or deionized water

6. Warnings and precautions for the users

This test should be carried out only by trained laboratory employees. The instruction for use must be strictly followed.

The standard solutions contain aflatoxin M1, particular care should be taken. Avoid contact of the reagent with the skin (use gloves).

Decontamination of the glassware and toxin-content solutions is best carried out using a sodium hypochlorite (bleach) solution (10 % (v/v)) overnight (adjust solution with HCl to pH 7).

This kit may contain hazardous substances. Please refer to the component safety information in the material safety data sheets (SDS) for this product, available online at www.r-biopharm.com.

Ensure the proper and responsible disposal of all reagents and materials after their use. For disposal, please adhere to national regulations.

7. Storage instructions

Store the kit at 2 - 8 °C (35 - 46 °F). Do not freeze any test kit components.

Return any unused microwells to their original foil bag, reseal them together with the desiccant provided and further store at 2 - 8 °C (35 - 46 °F).

Aflatoxins are light sensitive. Therefore, avoid exposure to direct light.

The reddish substrate/chromogen is light sensitive. Therefore, avoid exposure to direct light.

Do not use the test kit after the expiration date (see test kit label).

Do not interchange individual reagents between kits of different lot numbers.

8. Indication of instability or deterioration of reagents

– Bluish coloration of the reddish substrate/chromogen prior to addition in the wells
– Extinction less than 0.8 ($E_{450\ nm} < 0.8$) for zero standard

9. Sample preparation

The samples should be stored in a cool place, protected against light.

9.1 Milk

- Centrifuge milk samples for degreasing: 10 min / 3500 g / 10 °C (50 °F) (if a refrigerated centrifuge is not available, chill sample to 10 °C (50 °F) prior to centrifugation)
- After centrifugation, remove upper cream layer completely by aspirating through a pasteur pipette
- Use the skimmed milk (= defatted supernatant) directly in the test (100 µl per well)

9.2 Milk powder

The amount of sample can be adjusted. It should be noted that the sample has to be representative and that the ratio of sample to distilled water remains the same.

- Weigh in 10 g milk powder and fill up to 100 ml with distilled water
- Suspend completely by shaking or stirring
- Continue with the preparation of milk as described in capture 9.1

Remarks

If a further dilution is required, use sample buffer (see capture 4.) for the dilution.

10. Test procedure

10.1 Test preparation

Bring all reagents to room temperature (20 - 25 °C / 68 - 77 °F) before use.

As **washing buffer,** a PBS tween buffer is needed. Please use the wash buffer salt (see capture 4.) contained in the kit. Dissolve the entire wash buffer salt in one liter of distilled water. The ready to use buffer expires after approx. 4 - 6 weeks at 2 - 8 °C (36 - 46 °F).

> **Alternative:** Dissolve the contents of the pouch in 100 ml of distilled water to obtain a 10fold concentrated buffer. Use 1 part of this concentrate and dissolve with 9 parts of distilled water to obtain the ready to use buffer. This solution expires after approx. 8 - 12 weeks, store at room temperature (20 - 25 °C / 68 - 77 °F).

Unused reagents should be immediately stored at 2 - 8 °C.

10.2 Test procedure

Carefully follow the recommended washing procedure to obtain unambiguous results. Do not allow wells to dry between work steps.

1. Insert a sufficient number of wells into the microwell holder for all standards and samples to be run in duplicate. Record standard and sample positions. Do not use more than 4 strips (32 wells) at a time when testing milk powder in single determination.
2. Pipette 100 µl of antibody in duplicate to the wells, mix gently by shaking the plate manually and incubate for 15 min at room temperature (20 - 25 °C / 68 - 77 °F).
3. Pour the liquid out of the wells and tap the microwell holder upside down vigorously (three times in a row) against absorbent paper to ensure complete removal of liquid from the wells. Fill all wells with 250 µl wash buffer (see chapter 10.1) and pour out the liquid again. Repeat the washing procedure two times.
4. Pipette 100 µl standard or prepared sample to separate duplicate wells. Mix gently by shaking the plate manually and incubate for 30 min at room temperature (20 - 25 °C / 68 - 77 °F) in the dark.
5. Pour the liquid out of the wells and tap the microwell holder upside down vigorously (three times in a row) against absorbent paper to ensure complete removal of liquid from the wells. Fill all the wells with 250 µl wash buffer (see capture 10.1) and pour out the liquid again. Repeat the washing procedure two times.
6. Pipette 100 µl of conjugate. Mix gently by shaking the plate manually and incubate for 15 min at room temperature (20 - 25 °C / 68 - 77 °F) in the dark.
7. Pour the liquid out of the wells and tap the microwell holder upside down vigorously (three times in a row) against absorbent paper to ensure complete removal of liquid from the wells. Fill all the wells with 250 µl wash buffer (see capture 10.1) and pour out the liquid again. Repeat the washing procedure two times.
8. Pipette 100 µl of substrate/chromogen to each well. Mix gently by shaking the plate manually and incubate for 15 min at room temperature (20 - 25 °C / 68 - 77 °F) in the dark.

9. Pipette 100 µl of the stop solution to each well. Mix gently by shaking the plate manually and measure the absorbance at 450 nm. Read within 15 min after addition of stop solution.

11. Evaluation

A specific software, the **RIDASOFT® Win.NET** (Z9996FF), is available for evaluation of the RIDASCREEN® enzyme immunoassays. The course of the standard curve is shown in the Quality Assurance Certificate (certificate of analysis) enclosed in the test kit.

The calculation should be done by use of a cubic spline function.

Remark for the calculation without software:

$$\frac{\text{absorbance standard (or sample)}}{\text{absorbance zero standard}} \times 100 = \%\ \text{absorbance}$$

The zero standard is thus made equal to 100 % and the absorbance values are quoted in percentages. The values calculated for the standards are entered in a system of coordinates on semilogarithmic graph paper against the aflatoxin M1 concentration [ng/l].

12. Result interpretation

In order to obtain the aflatoxin M1 concentration in ng/l (ng/kg) actually contained in a sample, the concentration read from the calibration curve must be further multiplied by the corresponding dilution factor. When working in accordance with the regulation stated, the dilution factors are as follows:

milk 1
milk powder (referring to dissolved milk).....1
milk powder (referring to g-weight) 10

13. Further application notes

- Sample preparation for butter
- Sample preparation for cheese

Further product information and application requests, please contact your local distributor or R-Biopharm at this address: sales@r-biopharm.de.

Version overview

Version number	Chapter and title
2011-10-21	Release version
2020-05-08	General revision
2021-02-02	General revision 9.2 Change of component name of „sample dilution buffer“ into corrected name „sample buffer“

Explanation of symbols

- General symbols:

 Follow the instructions for use

 Batch number

 Expiry date (YYYY-MM)

 Storage temperature

 Article number

 Number of test determinations

 Manufacturing date (YYYY-MM)

 Manufacturer + address

Disclaimer

The user assumes all risk in using R-Biopharm AG´s products and services.

R-Biopharm AG will warrant that its products and services meet all quality control standards set by R-Biopharm AG, and R-Biopharm AG will, at its option, replace or repair any components, product or repeat services which prove to be defective in workmanship or material within product specific warranty periods or expiration dates and which our examination shall disclose to our satisfaction to be defective as such.

This warranty is expressly in lieu of all other warranties, expressed or implied, as to quality, description, fitness for any particular purpose, merchantability, productiveness, or any other matter. R-Biopharm AG shall be in no way responsible for the proper use of its products and hereby disclaims all other remedies, warranties, guarantees or liabilities, expressed or implied, arising by law or otherwise, and it shall have no liability for any lost profits or damage, direct, indirect or otherwise, to person or property, in connection with the use of any of its products or services.

This warranty shall not be extended, altered or varied except by a written instrument signed by an authorized representative of R-Biopharm AG.

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