RIDASCREEN® Aflatoxin Total

REF R4701

Enzymimmunoassay zur quantitativen Bestimmung von Aflatoxin

Enzyme immunoassay for the quantitative determination of aflatoxin

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In vitro Test

Lagerung bei 2 - 8 °C
Storage at 2 - 8 °C (36 - 47 °F)

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RIDASCREEN® Aflatoxin Total

Brief information

RIDASCREEN® Aflatoxin Total (Art. No. R4701) is a competitive enzyme immunoassay for the quantitative determination of aflatoxins in corn, barley, rice, wheat and feed (see chapter 1. Intended use).

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

Sample preparation:	grinding, extraction, filtration/centrifugation and dilution
Time requirement:	sample preparation (for 10 samples)... approx. 30 min test implementation (incubation time)..... 45 min
Limit of detection: (depending on matrix)	Corn .....2.40 µg/kg (ppb) Barley ..... < 1.75 µg/kg Rice…….....< 1.75 µg/kg Wheat .....< 1.75 µg/kg Feed.....7.80 µg/kg
Recovery rate: (corresponding to the standard substance)	in naturally contaminated samples (Ø) (Trilogy®) Corn .....approx. 125 % Feed.....approx. 91 % Brown Rice…….....approx. 118 % Wheat……approx. 134 % in artificially contaminated samples (Ø) Rice……approx. 136 % Wheat…….. ..approx. 133 % Barley ……...approx. 124 % Feed ……...approx. 145 %

Note: The assay was adjusted using naturally contaminated samples. Deviations in the recovery of spiked samples are possible.

Specificity:	Aflatoxin B1	100 %
	Aflatoxin B2	approx. 27 %
	Aflatoxin G1	approx. 89 %
	Aflatoxin G2	approx. 15 %
	DON	≤ 0.02 %
	Fumonisin B1	≤ 0.02 %
	Ochratoxin A	≤ 0.02 %
	T2-Toxin	≤ 0.02 %
	Zearalenone	≤ 0.02 %

The specificity of the RIDASCREEN® Aflatoxin Total 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 brochure. It lists minimum standards and conditions that are required when using test kits of R-Biopharm AG to perform ELISA analysis. The brochure can be retrieved, printed and downloaded from the website https://food.r-biopharm.com/media/technical-guides/.

Related product and accessories for aflatoxin determination

RIDASCREEN® Aflatoxin B1 30/15 (Art. No. R1211)
RIDASCREEN® FAST Aflatoxin (Art. No. R5202)
RIDASCREEN® FAST Aflatoxin SC (Art. No. R9002)
RIDA® QUICK Aflatoxin RQS (Art. No. R5208)
RIDA® QUICK Aflatoxin RQS ECO (Art. No. R5209)
RIDA® Aflatoxin column (Art. No. R5001 / R5002)
Trilogy® Liquid Standard Aflatoxin B1, B2, G1, G2
(Art. No. TAS-MM11LA1-10)
Trilogy® Liquid Standard Aflatoxin B1 (Art. No. TAS-M11LA1-10)
Trilogy® Dried Standard Aflatoxin B1, B2, G1, G2
(Art. No. TAS-MM11DA1-10)
Trilogy® Dried Standard Aflatoxin B1 (Art. No. TAS-M11DA1-10)
Trilogy® QC Material Aflatoxin (Art. No. TQC-M1111-100)

1. Intended use

RIDASCREEN® Aflatoxin Total is a competitive enzyme immunoassay for the quantitative determination of aflatoxins in corn, barley, rice, wheat and feed.

2. General information

Aflatoxins are secondary metabolites of the fungi species *Aspergillus flavus*, *parasiticus* und *nomius*. These fungi occur in humid tropical areas and the contamination of vegetable food takes place in the cultivable countries. Aflatoxins belong to the strongest natural occurring cancerogenic substances. Aflatoxin B_1 which is mostly found together with the aflatoxins B_2, G_1 and G_2 is the one with the highest toxic importance. It is found above all in corn, peanuts, brazil nuts, cotton seed and pistachios.
Due to the toxicity of these mycotoxins maximum levels for aflatoxin B1 and total aflatoxins for food and feed apply in EU countries.

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 antibodies. Standards or the sample solutions, aflatoxin-enzyme conjugate and anti-aflatoxin antibodies are added. Free and enzyme conjugated aflatoxin compete for the aflatoxin antibody binding sites (competitive enzyme immunoassay). At the same time, the anti-aflatoxin antibodies are also bound by the immobilized capture antibodies. Any unbound enzyme conjugate is then removed in a washing step. Substrate/chromogen solution 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 performed photometrically at 450 nm; the absorption is inversely proportional to the aflatoxin concentration in the sample.

4. Reagents provided

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

Component	Cap color	Format		Volume
Microtiter plate M	-	Ready to use		96 wells
Standard 1	White	Ready to use	0 µg/L	1.3 mL
Standard 2	White	Ready to use	0.05 µg/L	1.3 mL
Standard 3	White	Ready to use	0.15 µg/L	1.3 mL
Standard 4	White	Ready to use	0.45 µg/L	1.3 mL
Standard 5	White	Ready to use	1.35 µg/L	1.3 mL
Standard 6	White	Ready to use	4.05 µg/L	1.3 mL
Wash buffer salt Tween		Dissolve the salt		
Conjugate	Red	Ready to use		6 mL
Antibody	Black	Ready to use		6 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

- Gloves
- Scale (measurement range at least up to 50 g and precision of ± 0.01 g)
- Laboratory mincer / grinder, mortar, ultra-turrax or homogenizer
- Graduated cylinder (plastic or glass) 100 ml
- Graduated pipettes
- Shaker; optional: vortexer
- Filter funnel and 50 mL flask
- Filter paper: Whatman No. 1 or equivalent
- Optional: centrifuge + centrifugal vials with cap
- Variable 20 - 200 µL and 200 - 1000 µL micropipettes
- If necessary: 8-channel or multistepper pipette for 50 - 100 µL
- Microtiter plate spectrophotometer (450 nm)
- Optional: RIDASOFT® Win.NET (Art. No. Z9996FF)

5.2 Reagents

- Distilled water (dist. water) or deionized water
- 70 % methanol solution: mix 70 mL methanol (100 %) with 30 mL distilled water

6. Warnings and precautions for the users

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

The standards contain aflatoxin B_1. Particular care should be taken. Avoid contact of the reagent with the skin (use gloves).

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

This kit may contain hazardous substances. For hazard notes on the contained substances please refer to the appropriate material safety data sheets (SDS) for this product, available online at www.r-biopharm.com.

Do not reuse wells of the microtiter strips (coated microtiter, see chapter 10.2.). Use separate pipette tips for each standard and each sample extract to avoid cross contamination.

All reagents and materials must be recovered or disposed after use at customers own responsibility according to the protection of human health and the environment. Please observe the applicable national regulations concerning waste disposal (e.g. Waste Management Act, Regulations on Dangerous Chemicals, etc.).

7. Storage instructions

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

To avoid moisture inside the wells, open the foil bag for withdrawal of microwells only after having reached room temperature (20 - 25 °C / 68 - 77 °F).

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).

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 test implementation
- 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.

A representative sample (according to accepted sampling techniques) should be ground and thoroughly mixed prior to proceeding with the extraction procedure (recommended particle size: 500 µm).

Bring all reagents and samples to room temperature (20 - 25 °C / 68 - 77 °F) before use and perform the sample preparation at room temperature.

- Weigh 5 g of the ground and homogenized sample into a suitable container and add 25 mL of 70 % methanol*)
- Mix for 10 min at room temperature by shaking or vortexing
- Filter the extract through a Whatman No. 1 filter (or equivalent) or centrifuge (10 min / 3500 g / room temperature)
- Dilute 100 µL of the filtrate/supernatant with 600 µL distilled water
- Use 50 µL of the diluted filtrate/supernatant per well in the test

*) Sample size may be increased if required, but the volume of methanol/water must be adapted accordingly, e.g. 10 g in 50 mL of 70 % methanol.

Remark:

If the aflatoxin concentration is expected to exceed 120 µg/kg (ppb) further dilutions are necessary. For this use distilled water containing 10 % methanol, e.g. 9 mL distilled water + 1 mL methanol (100 %). Please note that any kind of sample being used in the assay has to be provided in distilled water with 10 % methanol.

R-Biopharm can provide you different applications for RIDASCREEN® Aflatoxin Total (Art. No. R4701) in combination with RIDA® Aflatoxin column (Art. No. R5001/R5002) upon request. **Please contact your local distributor or** sales@r-biopharm.de

10. Test procedure

10.1 Test preparation

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

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

Alternative: Dissolve the contents of the envelope in 100 mL of distilled water to obtain a 10-fold concentrated washing buffer. This 10-fold concentrate expires after approx. 8 weeks when stored at room temperature (20 - 25 °C / 68 - 77 °F). Use 1 part of this concentrate and dissolve with 9 parts of distilled water to obtain the ready to use wash buffer.

Components should be stored immediately at 2 - 8 °C (35 - 46 °F) when no longer required.

10.2 Test procedure

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

It is recommended to pipette the conjugate, the antibody, the substrate/chromogen and the stop solution with a multi-channel or stepper pipette to avoid a time shift over the plate.

Avoid direct sunlight during all incubations. Therefore cover the microtiter plates.

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.
2. Add 50 µL of each standard or sample (prepared according to chapter 9.) in duplicate to the wells. Use new pipette tips for each standard or sample.
3. Add 50 µL of the conjugate to each well.
4. Add 50 µL of the antibody to each well, 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 out the liquid of the wells and tap the microwell holder upside down vigorously (three times) on absorbent paper to ensure complete removal of liquid from the wells. Fill all the wells with 250 µL wash buffer (see

chapter 10.1) and pour out the liquid as before. Repeat two more times (a total of three wash cycles).

6. Add 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.
7. Pipette 100 µL of stop solution into each well. Mix gently by shaking the plate manually and measure the extinction at 450 nm. Read within 30 min after addition of stop solution.

11. Evaluation

Special software, **RIDASOFT® Win.NET (Art. No. Z9996FF)**, is optional available for evaluation of the RIDASCREEN® enzyme immunoassays. The evaluation should be done using the Cubic Spline function.

For the evaluation it should be clarified, that all quality criteria are fulfilled for the current test run. The course of the standard curve is shown in the Quality Assurance Certificate (certificate of analysis) enclosed in the test kit.

Remark for the calculation without software:

$$\frac{\text{absorbance standard (or sample)}}{\text{absorbance zero standard}} \times 100 = B/B_0\ (\%)$$

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 semilogarithmic against the aflatoxin concentration [µg/kg].

In order to obtain the aflatoxin concentration in µg/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:

Cereals and feed 35

12. Result interpretation

Results between LoD and LoQ indicate a low mycotoxin concentration in the sample. Calculated result show a high uncertainty in this area due to the method’s high variation below LoQ. Therefore, such results should not be reported with a quantitative value, but qualitative as “< LoQ”.

A result below the LoD does not exclude a mycotoxin contamination below the detection limit of the assay. The result should be reported accordingly.

A further dilution and new detection of samples is recommended for absorbance values ($A_{450\ nm}$) > standard 6. In case of a further dilution, the additional dilution factor must be taken into account when calculating the mycotoxin concentration.

13. Limits of the method

Test results may vary depending on the sample matrix, the actual test procedure and the laboratory environment.

Detection and quantification limits depend on the respective sample matrix, the degree of processing and the extraction method.

An incorrect weight of the sample to be analyzed will have a 1:1 effect on the measurement result (e.g. a 10 % higher concentration is measured with a weigh in of +10 %). A sufficient accuracy is given with a fluctuation of max. ±1 %.

14. Recommendation

In order to ensure a high analytical performance we recommend to analyze each sample material in duplicates. Each laboratory may decide to perform the test in single determinations after a qualified risk management analysis. This has no influence on the function of the test kit. However, it should be noted that this increases the risk of overlooking errors in the performance of the test (e.g. pipetting errors). Moreover, a higher result variation will occur when pipetting in single determinations.

In order to ensure a high analytical performance we recommend:

- Pre-flush pipette tips prior to pipetting.
- Carry along test controls for quality control. Mycotoxin-free and mycotoxin containing samples should be used.
- To contact sales@r-biopharm.de if automates (e.g. ThunderBolt® / Bolt™) are used.

15. Further application notes

Further application notes are available on request.

For further product information and applications, please contact your local distributor or R-Biopharm at this address: sales@r-biopharm.de.

Version overview

Version number	Chapter and title
2007-07-06	Release version
2009-11-12 2010-11-18	General revision
2016-09-09	Consideration of new label designations
2022-11-14	Current version General revision Changes made: – Additions in the chapters “Brief Information”, “Intended Use”, 5, 6, 7, 8, 9, 10 and 11 – Chapters 12 - 15 added

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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