RIDASCREEN® Aflatoxin B1 30/15

Art. No. R1211

Enzymimmunoassay zur quantitativen Bestimmung von Aflatoxin B1

Enzyme immunoassay for the quantitative analysis of Aflatoxin B1

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In vitro Test
Lagerung bei 2 - 8 °C
Storage at 2 - 8 °C

Für weitere Fragen stehen Ihnen gerne zur Verfügung:

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RIDASCREEN® Aflatoxin B1 30/15

Brief information

RIDASCREEN® Aflatoxin B1 30/15 (Art. No. R1211) is a competitive enzyme immunoassay for the quantitative determination of aflatoxin B1 in cereals and feed. All reagents required for the enzyme immunoassay - including standards - are contained in the test kit.
The test kit is sufficient for 96 determinations (including standards).
A microtiter plate spectrophotometer is required for quantification.

Sample preparation:	grinding, extraction, filtration and dilution
Time requirement:	sample preparation (for 10 samples) .....approx. 30 min test implementation (incubation time) .....45 min
Detection limit: (corresponding to the standard substance)	Cereals ..... 1 µg/kg (ppb) Soy.....1,7 µg/kg Feed (representative samples, e.g. cattle/pig/poultry/ horse/rabbit feed) .....4 µg/kg Dry cat food .....2 µg/kg
Recovery rate: (corresponding to the standard substance)	approx. 93% mean recovery rate for naturally contaminated corn reference materials
Specificity:	Aflatoxin B1 ..... 100 % Aflatoxin B2 ..... approx. 13 % Aflatoxin G1 ..... approx. 29 % Aflatoxin G2 ..... approx. 3.2 % Aflatoxin M1 ..... approx. 1.5 %

The specificity of the RIDASCREEN® Aflatoxin B1 30/15 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 (GEP) – manual in the respective version. Such lists minimum standards concerning the framework conditions when using test kits of R-Biopharm AG and performing ELISA analysis. The manual can be retrieved, printed and downloaded from the website www.r-biopharm.com/products/food-feed-analysis.

1. Intended use

RIDASCREEN® Aflatoxin B1 30/15 is a competitive enzyme immunoassay for the quantitative determination of aflatoxin B1 in cereals and feed.

2. General

Aflatoxins are secondary metabolites of the fungi species *Aspergillus flavus*, *parasiticus* and *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 carcinogenic substances.
Aflatoxin B1 appears nearly in all cases together with Aflatoxin B2, G1 and G2 and it is the analyte with the highest toxic significance. It is commonly found 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 microtiter wells are coated with capture antibodies directed against anti-aflatoxin antibodies.
Aflatoxin standards or sample solutions, aflatoxin enzyme conjugate and anti-aflatoxin antibodies are added. Free aflatoxin and aflatoxin enzyme conjugate 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 is added to the wells, 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 absorbance is inversely proportional to the aflatoxin concentration in the sample.

4. Reagents provided

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

Component	Cap color	Format		Volume
Microtiter plate K	-	Ready to use		96 wells
Standard 1*	White	Ready to use	0 µg/l	1.3 ml
Standard 2*	White	Ready to use	1 µg/l	1.3 ml
Standard 3*	White	Ready to use	5 µg/l	1.3 ml
Standard 4*	White	Ready to use	10 µg/l	1.3 ml
Standard 5*	White	Ready to use	20 µg/l	1.3 ml
Standard 6*	White	Ready to use	50 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

*) The dilution factor 10 resulting from the sample preparation has already been considered. Therefore, the aflatoxin B1 concentrations of samples can be read directly from the standard curve.

5. Reagents required but not provided

5.1. Equipment

- grinder (mill)
- graduated cylinder (plastic or glass) 100 ml
- graduated pipettes
- filter funnel and 50 ml flask
- filter paper: Whatman No. 1 or equivalent
- 50 µl, 100 µl and 1000 µl micropipettes
- microtiter plate spectrophotometer (450 nm)
- optional: shaker, centrifuge

5.2. Reagents

- 70 % methanol solution: prepare 70 % methanol solution by mixing 70 ml methanol (100 %) with 30 ml distilled or deionized water
- distilled or deionized water

6. Warnings and precautions for the users

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

The standard solutions contain aflatoxin B1, particular care should be taken. Avoid contact of the reagent with the skin (use gloves).

Decontamination of glassware and aflatoxin B1 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. For hazard notes on the contained substances please refer to the appropriate material safety data sheets (MSDS) for this product, available online at www.r-biopharm.com.

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).

Aflatoxin B1 is light-sensitive. Therefore, protect the aflatoxin B1 standards from exposure to direct light.

The red stained substrate/chromogen solution is light sensitive, therefore, avoid exposure to direct light.

No quality guarantee is accepted after the expiration date on the kit label.

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

8. Indication of instability or deterioration of reagents

- any bluish coloration of the red stained substrate/chromogen prior to test implementation
- a value of less than 0.6 absorbance units ($A_{450\ nm} < 0.6$) for the zero standard

9. Preparation of samples

The samples should be stored in a cool place, protected from light.
Bring all reagents and samples to room temperature (20 - 25 °C / 68 - 77 °F) before use.
A representative sample (according to accepted sampling techniques) should be ground and thoroughly mixed prior to proceeding with the extraction procedure.

- weigh 5 g of ground and homogenized sample into a suitable container and add 25 ml of 70 % methanol *)
- shake vigorously for three minutes (manually or with shaker)
- filter the extract through Whatman No. 1 filter (or equivalent) or centrifuge (10 min / 3500 g / room temperature)
- dilute 1 ml of the obtained filtrate or clear supernatant with 1 ml of distilled or deionized water
- use 50 µl of the diluted filtrate 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 high aflatoxin concentrations are expected prepared samples need to be further diluted. Please note that the prepared samples have to be in methanol/water (35/65) solution when analyzed in the assay.

10. Test implementation

10.1. Preliminary comments

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

The aflatoxin B1 standards are provided ready to use. The dilution factor 10 for the sample has been considered when labeling. Therefore, the aflatoxin B1 concentration of samples can be read directly from the standard curve.

Return all reagents to 2 - 8 °C (35 - 46 °F) immediately after use.

10.2. Wash buffer

As **wash buffer** a PBS tween buffer is needed. Please use the wash buffer salt contained in the kit (see 4.). Dissolve the entire buffer salt in one liter of distilled

water. The ready to use wash buffer expires after approx. 4 - 6 weeks at 2 - 8 °C (36 - 46 °F).

Alternative: Dissolve the contents of the envelope in only 100 ml of distilled water to obtain a 10fold concentrated wash buffer. This solution expires after approx. 8 - 12 weeks, 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.

10.3. Test procedure

Accurate washing is very important. Reproducibility in any enzyme immunoassay is largely dependent upon the consistency with which the microwells are washed. Carefully follow the recommended washing procedure as outlined in the test procedure. Do not allow microwells to dry up totally and avoid prolonged intervals between the working steps.

Avoid direct sunlight during all incubation steps and incubate plates in the dark.

1. Insert a sufficient number of wells into the microwell holder for all standards and samples to be run. Record standard and sample positions.
2. Pipet 50 µl of standard or prepared sample into separate wells. Use a new pipette tip for each standard or sample.
3. Add 50 µl of conjugate to each well.
4. Add 50 µl of antibody to each well. Mix gently by shaking the plate manually and incubate for 30 min (+/- 1) at room temperature (20 - 25 °C / 68 - 77 °F).
5. Pour the liquid out of the wells and tap the microwell holder vigorously upside down against absorbent paper (three times in a row) to ensure complete removal of liquid from the wells. Fill all the wells with 250 µl wash buffer (see 10.2.). Empty the wells again. Repeat two more times.
6. Add 100 µl of substrate/chromogen to each well. Mix gently by shaking the plate manually and incubate for 15 min (+/- 1) at room temperature (20 - 25 °C / 68 - 77 °F).
7. Add 100 µl of stop solution to each well. Mix gently by shaking the plate manually and measure the absorbance at 450 nm. Read within 15 minutes after addition of stop solution.

11. Results

A specific software, the RIDA®SOFT Win.net (Art. Nr. R9996), is available for evaluation of the RIDASCREEN® enzyme immunoassays.

For sample analysis we recommend to perform double or multiple determinations. Please use the RIDA®SOFT Win.net cubic spline function for test evaluation.

The course of the standard curve is shown in the Quality Assurance Certificate enclosed in the test kit.

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 B1 concentration [µg/kg]. The aflatoxin B1 concentration in µg/kg corresponding to the absorbance of each sample can be read from the calibration curve.

For further information and applications please contact your local distributor of R-Biopharm AG (sales@r-biopharm.de).

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