RIDASCREEN® Ochratoxin A 30/15

REF R1312

Enzymimmunoassay zur quantitativen Bestimmung
von Ochratoxin A

Enzyme immunoassay for the quantitative determination
of ochratoxin A

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In vitro Test

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

R-Biopharm AG, An der neuen Bergstraße 17, 64297 Darmstadt, Germany
Phone: +49 (0) 61 51 81 02-0 / Fax: +49 (0) 61 51 81 02-20

Für weitere Fragen stehen Ihnen gerne zur Verfügung:

Please contact for questions and further information:

R-Biopharm AG Zentrale
Tel.: +49 (0) 61 51 - 81 02-0

Auftragsannahme
Fax: +49 (0) 61 51 - 81 02-20
E-Mail: orders@r-biopharm.de

Marketing & Vertrieb
E-Mail: info@r-biopharm.de

R-Biopharm AG switchboard
Phone: +49 (0) 61 51 - 81 02-0

Order department
Fax: +49 (0) 61 51 - 81 02-20
E-mail: orders@r-biopharm.de

Marketing & sales
E-mail: sales@r-biopharm.de

RIDASCREEN® Ochratoxin A 30/15

Brief information

RIDASCREEN® Ochratoxin A 30/15 (Art. No. R1312) is a competitive enzyme immunoassay for the quantitative analysis of ochratoxin A in corn, wheat, barley, rye, rice and feed.

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:	Extraction, centrifugation, dilution
Time requirement:	Sample preparation (for 10 samples)
	Corn, wheat, barley, rye, rice ..... approx. 20 min
	Feed ..... approx. 20 min
	Test implementation (incubation time)..... 45 min
Limit of detection: (depending on matrix)	Corn .....0.5 µg/kg (ppb)
	Wheat .....0.5 µg/kg (ppb)
	Barley.....0.4 µg/kg (ppb)
	Rye .....1.2 µg/kg (ppb)
	Rice.....0.8 µg/kg (ppb)
	Feed.....1.6 µg/kg (ppb)
Recovery rate: (corresponding to the Standard substance)	in artificially contaminated samples (Ø)
	Barley (FAPAS)..... 80 - 100 %
	Rye ..... 100 - 130 %
	Rice..... 75 - 130 %
	Feed..... 60 - 100 %
	in naturally contaminated samples (Ø)
	Corn ..... 90 - 130 %
	Wheat ..... 90 - 140 %
	Feed..... 50 - 100 %

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

Specificity:	Ochratoxin A	approx.100 %
	Ochratoxin C	approx. 37 %
	Ochratoxin B	approx. 2 %
	Ochratoxin α	approx. 9 %
	Ochratoxin ß	< 0.02 %
	Deoxynivalenol	< 0.02 %
	Zearalenon	< 0.02 %
	Aflatoxin B1	< 0.02 %
	Fumonisin B1	< 0.02 %
	Citrinin	< 0.02 %

The specificity of the RIDASCREEN® Ochratoxin A 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 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 ochratoxin A determination

RIDASCREEN®FAST Ochratoxin A (Art. No. R5402)
RIDA® Ochratoxin A column (Art. No. R1303)

1. Intended use

RIDASCREEN® Ochratoxin A 30/15 (Art. No. R1312) is a competitive enzyme immunoassay for the quantitative analysis of ochratoxin A in corn, wheat, barley, rye, rice and feed.

2. General information

The mycotoxin ochratoxin A is formed by fungi of the species *Aspergillus* and *Penicillium*. Apart from a marked nephrotoxicity, ochratoxin A displays hepatotoxic, teratogenic, carcinogenic and immunosuppressive properties.
There is a risk to human health not only through the intake of contaminated foods of vegetable origin, but also through foods of animal origin. Ochratoxin A has been detected in pig blood and kidneys, as well as in human blood and mother's milk.

3. Test principle

The basis of the test is the antigen-antibody reaction. The wells in the microtiter strips are coated with specific antibodies against ochratoxin A. Ochratoxin A standards or the sample solutions and enzyme conjugate are added. Free and enzyme-conjugated ochratoxin A compete for the ochratoxin A antibody binding sites (competitive enzyme immunoassay). Any unbound enzyme conjugate is then removed in a washing step. 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. The absorption is inversely proportional to the ochratoxin A 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	-	Ready to use		96 wells
ECO extractor	Transparent	**Concentrate**	**10 x**	2 x 120 mL
Standard 1	White	Ready to use	0 µg/L	1.3 mL
Standard 2	White	Ready to use	0.03 µg/L	1.3 mL
Standard 3	White	Ready to use	0.1 µg/L	1.3 mL
Standard 4	White	Ready to use	0.3 µg/L	1.3 mL
Standard 5	White	Ready to use	1 µg/L	1.3 mL
Standard 6	White	Ready to use	3 µg/L	1.3 mL
Wash buffer salt Tween*		**Dissolve the salt**		
Conjugate	Red	Ready to use		6 mL
Substrate/Chromogen Red Chromogen Pro	Brown	Ready to use		13 mL
Stop solution	Yellow	Ready to use		14 mL

5. Reagents/Equipment required but not provided

5.1 Equipment

- Gloves
- Scale (measurement range at least up to 50 g and precision of ± 0.01 g)
- Laboratory mill / mincer / grinder, mortar, ultra-turrax or homogenizer
- Centrifuge (at least 3,500 x g) + centrifugal vials with cap (e.g. 50 mL centrifuge tubes from Greiner Art. No. 227261)
- (Horizontal) shaker
- Vortex mixer
- Graduated cylinder (plastic or glass) 100 mL
- Variable 20 - 200 µL and 200 - 1000 µL micropipettes
- If necessary: 8-channel pipette for 50 µL and 100 µL
- Microtiter plate spectrophotometer (450 nm)
- Optional: RIDASOFT® Win.NET (Art. No. Z9996FF)

5.2 Reagents

- Distilled water (dist. water) or deionized 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 ochratoxin A. Particular care should be taken. Avoid contact of the reagent with the skin (use gloves).

Decontamination of the glassware and ochratoxin A 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 (SDS) for this product, available online at www.r-biopharm.com.

Do not reuse wells of the microtiter strips (coated microtiter plate, 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
- Value of less than 0.8 absorbance units ($A_{450\ nm} < 0.8$) 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).

9.1. Extraction buffer

For extraction, the diluted ECO extractor is needed. To obtain the ready-to-use ECO extractor, dilute the ECO Extractor (10x concentrate) 1:10 with distilled or deionized water. The diluted ECO Extractor is stable for one week at 2 - 8 ° C (36 - 46 °F). If turbidity occurs in the diluted ECO extractor (possibly caused by contamination), discard or do not use it.

9.2. Extraction of corn, wheat, barley, rice and feed

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 10 g of ground and homogenized sample into a suitable container (e.g. 125 mL bottle) and add 50 mL of diluted ECO extractor
- Vortex the sample briefly (10 seconds)
- Shake the sample vigorously for 5 minutes (manually or with shaker at 420 rpm)
- Centrifuge for 5 min at 3,500 g and room temperature (20 - 25 °C / 36 - 46 °F)
- Dilute 1 mL of the supernatant with 1 mL of ready-to-use wash buffer (sample buffer, see chapter 10.1.)
- Add 50 µL of the diluted supernatant per well in the assay

Note
Corn, wheat, rice and feed samples measured outside the measurement range of > 30 µg/kg (ppb) should be further diluted 1:10 (1 + 9) with ready-to-use wash buffer (see chapter 10.1.).

9.3. Extraction of rye

- Weigh 5 g of ground and homogenized sample into a suitable container (e.g. 125 mL bottle) and add 50 mL of diluted ECO extractor
- Vortex the sample briefly (10 seconds)
- Shake the sample vigorously for 5 minutes (manually or with shaker at 420 rpm)
- Centrifuge for 5 min at 3,500 g and room temperature (20 - 25 °C; 36 - 46 °F)
- Dilute 1 mL of the supernatant with 1 mL of ready-to-use wash buffer (sample buffer, see chapter 10.1.)
- Add 50 µL of the diluted supernatant per well in the assay

Note
Rye samples measured outside the measurement range of > 60 µg/kg (ppb) should be further diluted 1:10 (1 + 9) with ready-to-use wash buffer (see chapter 10.1.).

10. Test procedure

10.1 Test preparation

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

As **wash buffer and sample buffer** a PBS tween buffer is needed. Please use the buffer salt contained in the kit (see chapter 4.). Dissolve the entire buffer salt in one liter of distilled water. The ready-to-use wash buffer expires after approx. 4 weeks when stored 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. The 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 (1 + 9).

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. Carefully follow the recommended washing procedure as outlined in the test procedure. Reproducibility in any ELISA is largely dependent upon the consistency with which the microwells are washed.

It is recommended to pipette the conjugate, 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. Pipette 50 µL of each standard or sample (prepared according to chapter 9.) in duplicate to the wells; use a new pipette tip for each standard or sample.
3. Add 50 µL of the conjugate 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.

4. 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).
5. 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.
6. 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 15 min after addition of stop solution.

11. Evaluation

Special software, **RIDASOFT® Win.NET Food & Feed (Art. No. Z9996FF)**, is optional available for evaluation of the RIDASCREEN® enzyme immunoassays. The evaluation should be done for double determinations 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 fort he 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 ochratoxin A concentration [µg/L].

12. Result interpretation

In order to obtain the ochratoxin A concentration in µg/L (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:

Corn, wheat, barley, rice and feed…… 10
Rye …… 20

Results between LoD and LoQ indicate a low ochratoxin A 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 ochratoxin A 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 ochratoxin A concentration. Further dilutions should be made with the wash buffer.

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.

Technical limits of the test method are approached outside the designated measurement range resulting in higher variation. This may cause a switch of results between the different areas of the calibration curve especially at the test characteristic boundaries (LoD, LoQ, upper limit of measurement range).

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 with standard or sample extract prior to pipetting.
- Carry along test controls for quality control. Mycotoxin-free and mycotoxin containing (spiked) samples should be used.
- To do spike experiments to ensure an accurate and correct test procedure.
- 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
2019-11-28	Release version
2020-03-23	General revision
2023-02-02	Current version General revision and editorial changes Changes made: – 6. Warnings and precautions for the users: additions – Chapter 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.

R-Biopharm AG
Postanschrift / Postal Address:
An der neuen Bergstraße 17
64297 Darmstadt, Germany
Sitz / Corporate Seat: Pfungstadt
Tel.: +49 (0) 61 51 - 81 02-0
Fax: +49 (0) 61 51 - 81 02-40
E-mail: info@r-biopharm.de
www.r-biopharm.com

Vorsitzender des Aufsichtsrats /
Chairman of Supervisory Board:
Dr. Ralf M. Dreher
Vorstand / Board of Management:
Christian Dreher (Vorsitzender / Chairman),
Jochen Hirsch, Ute Salzbrenner,
Hans Frickel, Dr. Peter Schubert
Handelsregister / Commercial Register:
Amtsgericht Darmstadt HRB 8321