RIDASCREEN® Ochratoxin A 30/15

REF R1312

Enzymimmunoassay zur quantitativen Bestimmung von Ochratoxin A

Enzyme immunoassay for the quantitative analysis of ochratoxin A

In vitro Test

Lagerung bei 2-8 °C
Storage at 2-8 °C

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 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:	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: (naturally contaminated samples - TRILOGY®)	Corn	90 - 130 %
	Wheat	90 - 140 %
	Feed	50 - 100 %
Recovery rate: (spiked samples)	Barley (FAPAS)	80 - 100 %
	Rye	100 - 130 %
	Rice	75 - 130 %
	Feed	60 - 100 %

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

Specificity:

The specificity of the RIDASCREEN® Ochratoxin A 30/15 test was determined by analyzing the cross-reactivities to corresponding mycotoxins 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.

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 %

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 under the website www.r-biopharm.com/products/food-feed-analysis.

Related products

RIDASCREEN®FAST Ochratoxin A (R5402)
RIDA® Ochratoxin A column (R1303)
TRILOGY® Certified Liquid Standard Ochratoxin A (CTSL-520-5)
TRILOGY® Liquid Standard Ochratoxin A (TSL-504-5)
TRILOGY® Dried Standard Ochratoxin A (TS-503-5)

1. Intended use

RIDASCREEN® Ochratoxin A 30/15 is a competitive enzyme immunoassay for the quantitative analysis of ochratoxin A in corn, wheat, barley, rye, rice and feed.

2. General

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.

3. Reagents provided

Each kit contains sufficient materials for 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

*) The ready-to-use wash buffer (see preparation 10.1) is used as sample and wash buffer.

5. Materials required but not provided

5.1. Equipment:

- Microtiter plate spectrophotometer (450 nm)
- Centrifuge (≥ 3,500 g)
- (Horizontal) shaker
- Laboratory or grain mill
- Vortex mixer
- Graduated cylinder (plastic or glass) 100 ml
- Variable 50 µl - 100 µl and 1000 µl micropipettes

5.2. Reagents:

– 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 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 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).

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

No quality guarantee is accepted after expiry of the kit (see kit label).

The test kit can be regularly used at least up to the expiry date (indicated on the kit package), if stored correctly.

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

8. Indication of instability or deterioration of reagents

– Any bluish coloration of the reddish substrate/chromogen solution prior to test implementation
– A value of less than 0.8 absorbance units ($A_{450\ nm} < 0.8$) for the zero standard

9. Preparation of Samples

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

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 deionised 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.
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). We recommend to produce an additional replica per unknown sample and naturally contaminated sample.

– 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 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 with ready-to-use wash buffer (see 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 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 with ready-to-use wash buffer (see 10.1.).

10. Test implementation

10.1. Preliminary comments

1. Bring all reagents to room temperature (20 - 25 °C / 68 - 77 °F) before use and perform the test at room temperature.
2. Return all reagents to 2 - 8 °C (35 - 46 °F) immediately after use.

As wash buffer and sample buffer a PBS tween buffer is needed. Please use the 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 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 10fold concentrated washing buffer. The 10fold concentrate expires after approx. 8 - 12 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.

10.2. Test procedure

Carefully follow the recommended washing procedure. Do not allow microwells to dry up completely and avoid prolonged intervals between the working steps. Therefore, the processing should be done quickly. Carefully follow the recommended washing procedure as outlined in the test procedure. Reproducibility in any EIA is largely dependent upon the consistency with which

the microwells are washed. Not more than 6 strips per test run should be processed.

Avoid direct sunlight during all incubations. Therefore, covering the microtiter plates is recommended.

1. Insert a sufficient number of microtiter 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 the standard solutions or prepared sample to separate duplicate wells; use a new pipette tip for each standard or sample.
3. Add 50 µl of 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 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 10.1.) and pour out the liquid again. Repeat the washing procedure two times.
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. 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 special software, the RIDASOFT® Win.net (Art. No. Z9996), is available for evaluation of the RIDASCREEN® enzyme immunoassays. The calculation should be done in double determinations by use of a cubic spline function. The course of the standard curve is shown in the Quality Assurance Certificate enclosed in the test kit.

In order to obtain the ochratoxin A concentration in µg/kg (ppb) actually contained in a sample, the concentration read from the standard 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, rye, rice and feed…….. 10
Rye……20

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

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

The data corresponds to our present state of technology and provides information on our products and their uses. R-Biopharm makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. Defective products will be replaced. There is no warranty of merchantability of this product, or of the fitness of the product for any purpose. R-Biopharm shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.

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),
Dr. Hans Frickel, Jochen Hirsch, Dr. Peter Schubert
Handelsregister / Commercial Register:
Amtsgericht Darmstadt HRB 8321