RIDASCREEN® Zearalenon ECO

REF R1403

Enzymimmunoassay zur quantitativen Bestimmung von Zearalenon

Enzyme immunoassay for the quantitative determination of zearalenone

In vitro Test

Lagerung bei 2 - 8 °C
Storage at 2 - 8 °C (36 - 46 °F)

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RIDASCREEN® Zearalenon ECO

Brief information

RIDASCREEN® Zearalenon ECO (Art. No. R1403) is a competitive enzyme immunoassay for the quantitative determination of zearalenone in corn and wheat (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: homogenization, extraction, centrifugation and dilution

Time requirement: sample preparation (for 10 samples)... approx. 10 min
test implementation (incubation time)..... 45 min

Limit of detection (LoD):……7.5 µg/kg (ppb)

Limit of quantification (LoQ): .....20 µg/kg (ppb)

Specificity: Zearalenone……100 %

Please see the validation report for further information.

The specificity of the RIDASCREEN® Zearalenon ECO 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 LoD 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 zearalenone determination

RIDASCREEN®FAST Zearalenon (Art. No. R5502)
RIDASCREEN®FAST Zearalenon SC (Art. No. R5505)
RIDA®QUICK Zearalenon RQS (Art. No. R5504)
RIDA®ABSORBANCE 96 (Art. No. ZRA96FF)
RIDASOFT®Win.NET Food & Feed (Art. No. Z9996FF)
Trilogy® Certified reference materials and standards for zearalenone (ISO 17034)

1. Intended use

RIDASCREEN® Zearalenon ECO (Art. No. R1403) is a competitive enzyme immunoassay for the quantitative determination of zearalenone in corn and wheat.

2. General information

The mycotoxin zearalenone is formed by fungi of the genus *Fusarium*. Zearalenone is a phytohormone which displays, apart from its anabolic properties, mainly estrogenic effects. Because of its estrogenic properties, zearalenone may induce fertility disorders in animals with clinical signs of hyperestrogenism - an aspect of a disease which although reported mainly in hogs, is described in other species such as cow, horse and sheep. The potential health risk for humans induced by this mycotoxin, which is taken up with foods of vegetable or animal origin, is extensively discussed.

3. Test principle

The basis of the test is the antigen-antibody reaction. The microtiter wells are coated with capture antibodies directed against anti-zearalenone antibodies. Zearalenone standards or sample solutions, zearalenone enzyme conjugate and anti-zearalenone antibodies are added. Free zearalenone and zearalenone enzyme conjugate compete for the zearalenone antibody binding sites (competitive enzyme immunoassay). At the same time, the anti-zearalenone 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 made photometrically

at 450 nm. The absorbance is inversely proportional to the zearalenone 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
ECO extractor	Transparent	**Concentrate**	**10 x**	120 mL
Standard 1*	White	Ready to use	0 µg/L	1.3 mL
Standard 2*	White	Ready to use	7.5 µg/L	1.3 mL
Standard 3*	White	Ready to use	22.5 µg/L	1.3 mL
Standard 4*	White	Ready to use	67.5 µg/L	1.3 mL
Standard 5*	White	Ready to use	202.5 µg/L	1.3 mL
Standard 6*	White	Ready to use	607.5 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	-	13 mL
Stop solution	Yellow	Ready to use	-	14 mL

(* The dilution factor 30 for the sample preparation has already been considered. Therefore, the zearalenone concentrations of samples (µg/kg) can be read directly from the standard curve.

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) 25 mL
- Centrifuge (at least 3,500 x g) + centrifugal vials with cap (e.g. 50 mL centrifuge tubes from Greiner Art. No. 227261)
- Shaker (horizontal)
- Vortex
- Variable 20 - 200 µL and 200 - 1000 µL micropipettes
- If necessary: 8-channel pipette for 50 µL, 100 µL und 250 µL
- Microtiter plate spectrophotometer (450 nm)
- Optional: RIDA®ABSORBANCE 96 (Art. Nr. ZRA96FF)
- Optional: RIDASOFT® Win.NET Food & Feed (Art. No. Z9996FF)

5.2 Reagents

– Distilled water (dist. water) or deionized water

6. Warnings and precautions for the users

The test is only suitable within the scope of its intended use.

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

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

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 Test procedure). 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 (36 - 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 (36 - 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 standard 1

9. Sample preparation

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

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).

9.1 Preparation of components

The **diluted ECO extractor** is required for the extraction. ECO Extractor is available as a 10-fold concentrate and must therefore be diluted 1:10 (1 + 9) with deionized or distilled water before use (e. g. 100 mL concentrate + 900 mL dist. water). The diluted ECO Extractor has a shelf life of one week at 2 - 8 °C. If turbidity occurs in the diluted ECO Extractor (e.g. caused by contamination), it must be discarded.

For dilution and wash cycles, the PBS Tween buffer is needed; please use the enclosed wash buffer salt Tween (see chapter 4.). To prepare the buffer, dissolve the entire contents of the pouch in 1 L distilled water. The **dissolved wash buffer** can be stored for approximately 4 to 6 weeks at 2 - 8 °C.

> Alternative: Dissolve the contents of the pouch in 100 mL distilled water (10-fold concentrate). The solution can be stored for approximately 8 – 12 weeks at room temperature (20 - 25 °C). To prepare the ready-to-use solution, mix 1 part of the 10-fold concentrate with 9 parts of distilled water.

9.2 Preparation of corn and wheat samples

1. Weigh 5 g of ground and homogenized sample into a suitable vessel and add 25 mL of **diluted ECO extractor** *)
2. Vortex the sample briefly (approx. 10 seconds)
3. Shake the sample vigorously for 5 minutes manually or with an orbital shaker
4. Centrifuge at 3,500 g for 5 minutes at room temperature (20 - 25 °C / 36 – 46 °F)
5. Dilute the supernatant 1:6 (1 + 5) with **dissolved wash buffer**, e.g. 100 µL of supernatant + 500 µL dissolved wash buffer
6. Add 50 µL per well in the assay

*) The sample weight can be increased if required; however, the volume of diluted ECO Extractor must be adjusted proportionally — e.g. 10 g of sample in 50 mL of diluted ECO Extractor.

Note

Since the dilution factor of 30 is already incorporated into the standard curve, the factor is 1 after the sample preparation described above. For samples measured outside the calibration range (> 607.5 µg/kg), further dilution is recommended. Therefore, dilute the supernatant obtained after centrifugation (step 4) with diluted ECO Extractor, e.g., 1:x (200 µL supernatant + (x - 1) * 200 µL diluted ECO Extractor). Then, carry out the regular 1:6 final dilution of the diluted supernatant with dissolved wash buffer according to step 5. The additional dilution factor x must be taken into account accordingly in the calculation.

10. Test procedure

10.1 Test preparation

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

Components should be stored immediately at 2 - 8 °C (36 - 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. Pipette 50 µL of each standard or sample (prepared according to chapter 9) in duplicate to the wells, using a new pipette tip for each standard or sample.
3. Add 50 µL of conjugate into each well
4. Add 50 µL of antibody into each well. Gently mix by shaking the plate manually and incubate for 30 minutes 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 dissolved wash buffer (see chapter 9.1) and pour out the liquid as before. Repeat two more times (total of three wash cycles).
6. Add 100 µL of substrate/chromogen to each well and incubate for 15 min at room temperature (20 - 25 °C / 68 - 77 °F) in the dark.
7. Add 100 µL of stop solution into 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 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 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 certificate of analysis (CoA).

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 zearalenone concentration [µg/L].

12. Result interpretation

Results between LoD (Limit of Detection) and LoQ (Limit of Quantification) indicate a low zearalenone concentration in the sample. Calculated results show a high uncertainty in this area due to the method's higher 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 zearalenone contamination below the detection limit of the assay. The result should be reported accordingly: < LoD.

For further dilution and new measurement 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 zearalenone concentration.

Compared to the certificate, higher absorbance values ($A_{450\ nm}$) for the standard curve, especially for the zero standard, may be a result of insufficient washing or zearalenone contamination.

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.

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. Naturally and artificially (spiked) contaminated samples should be used.
- In case of extremely acidic or basic samples, adjust the sample's pH value (pH 6.5 - 7.5) to neutral prior to extraction.
- Analysis of artificially contaminated samples (spiked samples) shall be conducted to verify the accuracy and error-free execution of the assay.

15. Further information

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

Version overview

Version number	Chapter and title
2025-06-06	Release version

Explanation of symbols

General symbols:

 Follow the instructions for use

 Batch number

 Expiry date (YYYY-MM-DD)

 Storage temperature

 Article number

 Number of test determinations

 Manufacturing date (YYYY-MM-DD)

 Manufacturer + address

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