RIDASCREEN® Zearalenon

Enzymimmunoassay zur quantitativen Bestimmung von Zearalenon

Enzyme immunoassay for the quantitative analysis of zearalenone

Art. No.: R1401

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

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RIDASCREEN® Zearalenon

Brief information

RIDASCREEN® Zearalenon (Art. No.: R1401) is a competitive enzyme immunoassay for the quantitative analysis of zearalenone in cereals, feed, beer, serum and urine.
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:	cereals and feed: extraction, filtration and dilution beer: removing of CO_2 and dilution serum and urine: chromatographic purification by means of RIDA® C18 column (Art. No.: R2002)
Time requirement:	sample preparation (for 10 samples) Cereals and feed ..... approx. 20 min beer ..... approx. 10 min serum and urine ..... approx. 3.5 h test implementation (incubation time) ..... 2.5 h
Detection limit: (corresponding to the standard substance)	cereals and feed ..... approx. 1750 ppt beer ..... approx. 250 ppt serum and urine ..... approx. 50 ppt
Recovery rate: (corresponding to the standard substance)	in spiked cereals and feed samples ..... approx. 80 % (mean coefficient of variation of 15 %)
Specificity:	The specificity of the RIDASCREEN® Zearalenon test was established by analyzing the cross-reactivity to corresponding mycotoxins.

Zearalenone	100 %
α-Zearalenol	approx. 41.6 %
Zeranol (Zearalanol)	approx. 27.7 %
β-Zearalenol	approx. 13.8 %

1. Intended use

RIDASCREEN® Zearalenon is a competitive enzyme immunoassay for the quantitative analysis of zearalenone residues in cereals, feed, beer, serum and urine.

2. General

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, too.
The potential health risk for man 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 wells in the microtiter strips are coated with specific antibodies against zearalenone. Zearalenone standards or sample solutions and enzyme conjugate are added. Free and enzyme conjugated zearalenone compete for the zearalenone antibody binding sites (competitive enzyme immunoassay). Any unbound enzyme conjugate is then removed in a washing step. Enzyme substrate and chromogen are added to the wells and incubated. Bound enzyme conjugate converts the colorless 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 zearalenone concentration in the sample.

4. Reagents provided

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

1 x Microtiter plate with 96 wells (12 strips with 8 wells each)
coated with antibodies against zearalenone
6 x Standard solutions (1.3 ml each)
0 ppt (zero standard), 50 ppt, 150 ppt, 450 ppt, 1350 ppt, 4050 ppt
zearalenone in aqueous solution
1 x Conjugate (0.7 ml).....red cap
peroxidase conjugated zearalenone
concentrate
1 x Substrate (7 ml) .....green cap
contains urea peroxide
1 x Chromogen (7 ml) ..... blue cap
contains tetramethylbenzidine
1 x Stop solution (14 ml) ..... yellow cap
contains 1 N sulfuric acid
1 x Buffer 1 (50 ml) .....white cap
sample and conjugate dilution buffer

5. Materials required but not provided

5.1. Equipment:

- microtiter plate spectrophotometer (450 nm)
- grinder (mill)
- shaker
- glassware for preparing sample extract: filter funnel and 100 ml flask
- filter paper: Whatman No. 1 or equivalent
- centrifuge
- rotary evaporator or another equipment for evaporation of solvents
- pasteur pipettes
- graduated pipettes
- variable 20 µl - 200 µl and 200 - 1000 µl micropipettes

5.2. Reagents:

- methanol p.a.

additional for the preparation of serum/urine samples:

- Glucuronidase/arylsulfatase of Helix promatia (Merck, Art. No.: 4114)
- 50 mM sodium acetate buffer, pH 4.8
- 20 mM Tris buffer, pH 8.5 / methanol (80/20)
- RIDA® C18 column (Art. No.: R2002)

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 zearalenone, particular care should be taken. Avoid contact of the reagent with the skin (use gloves).

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

The stop solution contains 1 N sulfuric acid (R36/38, S2-26).

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 and reseal them together with the desiccant provided and further store at 2 - 8 °C (35 - 46 °F).

Zearalenone is light sensitive, therefore, avoid exposure to direct light.

The colorless chromogen is light sensitive, therefore, avoid exposure to direct light.

No quality guarantee is accepted after expiry of the kit (see 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 chromogen solution 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 against light.

9.1. Cereals and feed

A representative sample is triturated and thoroughly mixed in a mixer.

- weigh 5 g of ground sample into a suitable container and add 25 ml of methanol/water (70/30) *)
- shake vigorously for three minutes (manually or with shaker)
- centrifuge the extract: 10 min / 3500 g / room temperature (20 - 25 °C / 68 - 77 °F) or filter the extract through Whatman No. 1 filter
- dilute the supernatant or filtrate 1:7 (1+6) with sample dilution buffer (buffer 1) (e.g. 100 µl supernatant or filtrate + 600 µl buffer 1) **)
- use 50 µl of the diluted extract 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 methanol/water (70/30)

**) Diluted extracts of wheat samples may get turbid which can sometimes lead to a reduced recovery rate. Therefore we recommend to centrifuge the diluted extract at high speed (3 min / 20000 g / room temperature (20 - 25 °C / 68 - 77 °F)) and use 50 µl of the cleared supernatant in the test.

Remark:

The methanolic extract (supernatant or filtrate) can be stored at 2 - 8 °C (35 - 46 °F) for 2 weeks and at -20 °C (- 4 °F) for 2 months. Store the extract well sealed in glass vials (brown glass) and protected against light.

If results with higher zearalenone concentrations are expected, the sample solution has to be diluted further with sample dilution buffer (buffer 1).

9.2. Beer

- use a sufficient volume of beer sample and remove CO_2 until no formation of bubbles is visible (by stirring or filtration)
- then dilute the sample 1:5 (1+4) with sample dilution buffer (buffer 1) (e.g. 100 µl sample + 400 µl buffer 1)
- use 50 µl per well in the assay

Remark:

In the case of cloudy samples a sterile filtration of the sample is recommended before the sample is used in the assay!

9.3. Serum and urine

Preparation only for urine:

- dilute 0.5 ml urine with 3 ml 50 mM acetate buffer, pH 4.8
- add 8 µl of glucuronidase/arylsulphatase from Helix promatia
- incubate the solution for 3 h at 37 °C (98 °F)

3.5 ml hydrolyzed urine or 0.5 ml serum (without preparation) are purified by means of RIDA® C18 column (Art. No.: R2002):

- flow rate 1 drop/sec

- rinse the column with 3 ml methanol (100 %)
- equilibrate the column with 2 ml 20 mM Tris buffer, pH 8.5 / methanol (80/20; v/v)
- apply 3.5 ml hydrolyzed urine or 0.5 ml serum sample
- rinse the column with 2 ml 20 mM Tris buffer, pH 8.5 / methanol (80/20; v/v)
- rinse the column with 3 ml methanol (40 %)
- dry the column by an air or nitrogen-stream for 1 min
- elute sample slowly (flow rate 15 drops per min) with 1 ml methanol (80 %)
- evaporate eluate to dryness at maximum 60 °C (140 °F) (possibly under a weak nitrogen-stream under a chemical hood)
- redissolve the dried residue with 50 µl methanol, then add 450 µl sample dilution buffer (buffer 1) and mix thoroughly
- use 50 µl per well in the test

Remark:

Additional application notes for meat and milk are available on request. Please contact your local distributor.

10. Test implementation

10.1. Preliminary comments

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

The zearalenone enzyme conjugate (bottle with red cap) is provided as a concentrate. Since the diluted enzyme conjugate has a limited stability, only the amount which actually is needed should be reconstituted. Before pipetting, the enzyme conjugate should be shaken carefully. For reconstitution, the concentrate is diluted 1:11 (1+10) in buffer 1 (bottle with white cap, e.g. 200 µl concentrate + 2.0 ml buffer, sufficient for 4 microtiter strips).

10.2. Test procedure

Carefully follow the recommended washing procedure. Do not allow microwells to dry between working steps.

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.
3. Add 50 µl of the diluted enzyme conjugate to each well. Mix gently by shaking the plate manually and incubate for 2 h 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 distilled water and pour out the liquid again. Repeat the washing procedure two times.
5. Add 50 µl of substrate and 50 µl of chromogen 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.
6. Add 100 µl of the stop solution to each well. Mix gently by shaking the plate manually and measure the absorbance at 450 nm. Read within 30 minutes after addition of stop solution.

11. Results

A special software, the RIDA®SOFT Win (Art. No. Z9999), is available for evaluation of the RIDASCREEN® enzyme immunoassays.

For single determinations we recommend logit/log evaluation and for double or multiple determinations cubic spline should be used.

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 zearalenone concentration [ng/kg].

The zearalenone concentration in ng/kg corresponding to the absorbance of each sample can be read from the calibration curve.

In order to obtain the zearalenone concentration in ng/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
beer.....5
serum and urine 1