RIDASCREEN® DON

Enzymimmunoassay zur quantitativen Bestimmung von Deoxynivalenol

Enzyme immunoassay for the quantitative analysis of deoxynivalenol

Art. No.: R5906

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

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Location: Moldova

R-Biopharm AG, Darmstadt, Germany
Tel.: +49 (0) 61 51 81 02-0 / Telefax: +49 (0) 61 51 81 02-20

Anschrift:

R-Biopharm AG
An der neuen Bergstraße 17
D-64297 Darmstadt
www.r-biopharm.de

Für weitere Fragen stehen Ihnen gerne zur Verfügung:

Telefon:

Zentrale / Auftragsannahme	(0 61 51) 81 02-0

Telefax / E-Mail:

Auftragsannahme	(0 61 51) 81 02-20 orders@r-biopharm.de
Marketing	(0 61 51) 81 02-40 info@r-biopharm.de

RIDASCREEN® DON

Brief information

RIDASCREEN® DON (Art. No.: R5906) is a competitive enzyme immunoassay for the quantitative analysis of deoxynivalenol in cereals, malt, feed, beer and wort.
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.
Technicians need no specialized training to perform the RIDASCREEN® DON test, however, free support is offered from the distributor on request, if necessary.

Sample preparation:
cereals, malt, feed: extraction, filtration
beer: removing of CO_2
wort: without sample preparation

Time requirement:
sample preparation (for 10 samples)
cereals, malt, feed ..... approx. 10 min
beer ..... approx. 5 min
wort ..... none
test implementation (incubation time) ..... 45 min

Detection limit:
cereals, malt, feed ..... 18.5 ppb
beer ..... 3.7 ppb
wort ..... 3.7 ppb

Recovery rate:
in cereals, malt, feed, beer and wort ..... 85 - 110 %

Specificity:
The specificity of the RIDASCREEN® DON test was determined by analyzing the cross-reactivities to corresponding mycotoxins.

Deoxynivalenol ..... 100 %
3-Acetyldeoxynivalenol ..... > 100 %
15-Acetyldeoxynivalenol ..... approx. 19 %
Nivalenol ..... approx. 4 %
Fusarenon X ..... < 1 %
T-2 Toxin ..... < 1 %

1. Intended use

RIDASCREEN® DON is a competitive enzyme immunoassay for the quantitative analysis of DON in cereals, malt, feed, beer and wort.

2. General

Deoxynivalenol belongs to the trichothecene group of mycotoxins and is formed by fungi of the genus *Fusarium*. Deoxynivalenol often occurs in plant products particularly in cereals. More as 150 trichothecenes are known and the mycotoxins deoxynivalenol, 3-acetyl- and 15-acetyl-deoxynivalenol are the toxins most frequently occurring in Europe and Northern America. The toxin concentrations found in wheat, corn or rice are often in the ppm range. Due to their high cytotoxic and immunosuppressive properties these toxins pose a risk to human and animal health.

3. Test principle

The basis of the test is the antigen-antibody reaction. The microtiter wells are coated with capture antibodies directed against anti-deoxynivalenol antibodies. Deoxynivalenol standards or sample solutions, deoxynivalenol enzyme conjugate and anti-deoxynivalenol antibodies are added. Free deoxynivalenol and deoxynivalenol enzyme conjugate compete for the deoxynivalenol antibody binding sites (competitive enzyme immunoassay). At the same time, the deoxynivalenol antibodies are also bound by the immobilized capture antibodies. Any unbound enzyme conjugate is then removed in a washing step. After 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 deoxynivalenol concentration in the sample.

4. Reagents provided

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

1 x Microtiter plate with 96 wells (12 strips with 8 removable wells each)
coated with capture antibodies
5 x Standard solutions (1.3 ml each)
0 ppb (zero standard), 3.7 ppb, 11.1 ppb, 33.3 ppb, 100 ppb
deoxynivalenol in water
ready to use
1 x Conjugate (6 ml) ..... red cap
peroxidase conjugated deoxynivalenol
ready to use
1 x Anti-deoxynivalenol antibody (6 ml) ..... black cap
monoclonal
ready to use
1 x Substrate/Chromogen (10 ml) ..... brown cap
stained red
contains tetramethylbenzidine
1 x Stop solution (14 ml) ..... yellow cap
contains 1 N sulfuric acid
1 x Washing buffer (salt)
for preparation of a 10 mM phosphate buffer (pH 7.4)
contains 0.05 % Tween 20

5. Reagents required but not provided

5.1. Equipment:

- microtiter plate spectrophotometer (450 nm)
- graduated cylinder (plastic or glass) 100 ml, 1 liter,
- glassware for preparing sample extract: filter funnel and 50 ml flask
- grinder (mill)
- optional: shaker
- filter paper: Whatman No. 1 or equivalent
- variable 20 µl - 200 µl and 200 - 1000 µl micropipettes

5.2. Reagents:

- distilled or deionized water

6. Warnings and precautions for the users

The standards contain deoxynivalenol, so avoid contact of the reagent with the skin (use gloves).

Decontamination of the glassware and deoxynivalenol solutions is best carried out using a sodium hypochlorite (bleach) solution (10:90; 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, reseal them together with the desiccant provided and further store at 2 - 8 °C (35 - 46 °F).

The 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 reddish substrate/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.

A representative sample (according to accepted sampling techniques) should be ground and thoroughly mixed prior to proceeding with the extraction procedure.

9.1. Cereals, malt and feed

- weigh 5 g of ground sample and add it to a suitable container with 25 ml of distilled water *)
- shake vigorously for three minutes (manually or with shaker)
- filter the extract through Whatman No. 1 filter
- use 50 µl of the filtrate per well in the test

*) sample size may be increased if required, but the volume of water must be adapted accordingly, e.g.: 25 g in 125 ml distilled water or 50 g in 250 ml distilled water

9.2. Beer

- use a sufficient volume of beer sample and remove excessive CO_2 until no formation of bubbles is visible (by stirring or filtration)
- use 50 µl of CO_2 -free sample per well in the assay

In the case of cloudy beer samples sterile-filtration of the sample after removing the excessive CO_2 is recommended before the sample is used in the assay.

9.3. Wort

- use 50 µl of undiluted sample per well in the assay

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

10. Test implementation

10.1. Preliminary comments

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

A PBS-Tween buffer is needed as **washing buffer**, please use the washing buffer salt contained in the kit (see 4.). The contents is dissolved in one liter of distilled water. The ready to use washing buffer expires after apprx. 4 - 6 weeks at 2 - 8 °C (35 - 46 °F).

Alternatively: Dissolve the contents of the pouch in 100 ml of distilled water to obtain a 10fold concentrated washing buffer. This solution expires after approx. 8 - 12 weeks, stored at room temperature (20 - 25 °C / 68 - 77 °F).
Use one part of this concentrate and dissolve with 9 parts of distilled water to obtain the ready to use washing buffer.

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 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 to separate wells; use a new pipette tip for each standard or sample.
3. Add 50 µl of enzyme conjugate (red cap) to the bottom of each well.
4. Add 50 µl of the anti-deoxynivalenol antibody (black cap) to each well. Mix gently by shaking the plate manually and incubate for 30 min at room temperature (20 - 25 °C / 68 - 77 °F).
5. Dump the liquid out of the wells into a sink. Tap the microwell holder upside down onto a clean filter towel (three times in a row) to remove all remaining liquid from the wells. Using a multichannel pipette, fill the wells each with 250 µll of washing buffer (see 10.1.). Empty the wells again and remove all remaining liquid. Repeat the washing step two more times.
6. Add 100 µl of substrate/chromogen (brown cap) 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.
7. Add 100 µl of stop solution (yellow cap) to each well. Mix gently by shaking the plate manually and measure the absorbance at 450 nm. Read within 10 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 deoxynivalenol concentration [µg/kg].

In order to obtain the deoxynivalenol concentration in 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:

cereals, malt, feed	5
beer	1
wort	1