



Deoxynivalenol ELISA Assay Kit

(For Research Use Only)

Instructions

**Competitive enzyme immunoassay for the
quantitative detection of Deoxynivalenol**

Catalog number: BTDOEK-001

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Deoxynivalenol ELISA Assay Kit

Competitive enzyme immunoassay for quantitative detection of Deoxynivalenol (DON) in food and feed crop (cereals).

For in-vitro application

- 96 Test cavities
- Assay time: 45 min (15+15+15 min)
- Lower detection limit:

Wheat flour	12 ppb
oats	15 ppb
- Recovery rate: 80 – 110 %
- Cross reactivity:

15-Acetyldeoxynivalenol:	>100 %
3-Acetyldeoxynivalenol:	<1 %
T2-Toxin:	<1 %
Nivalenol:	<1 %

Storage: 2 – 8 °C

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Please read the directions for use carefully before carrying out the test!



Introduction

Deoxynivalenol (DON), also known as Vomitoxin, is a type of mycotoxin (toxic mould fungus). Chemically it belongs to type B trichothecenes. The mycotoxin DON is formed as a poisonous metabolite from the *Fusarium* genus, i.e. *F. culmorum* and *F. graminearum*.

DON enters into the human food chain via contamination of plant-based foods. It is abundant in grains such as wheat, barley, oats, rye, maize and processed grains such as malt, beer and bread. The main toxin production by *Fusarium* species happens in the field. Climate conditions have a big influence on contamination. The harvest and storage conditions play also an important role in DON contamination.

DON is a potent inhibitor of protein synthesis and thus acts generally cause cell damage. The mycotoxin DON has been connected with incidents of mycotoxicoses in both humans and farm animals.

Purpose of use

The Deoxynivalenol ELISA Assay Kit allows the quantitative determination of Deoxynivalenol in food and feed crop (cereals). For further applications the user is encouraged to verify the validity of the tests themselves or contact the manufacturer.

The test is easy to use, requires no complex instrumental equipment, and delivers the sensitive results quickly. After a sample preparation to isolate the Deoxynivalenol, a maximum of 36 samples in duplicate can be tested in 45 minutes with the Deoxynivalenol ELISA. The sample extraction with water is described in the following. Alternatively, Deoxynivalenol can also be isolated with the help of immunoaffinity chromatography.

Principle of the method

The Deoxynivalenol ELISA is a competitive enzyme immunoassay. The assay is carried out in a microplate, of which the cavities have already been pre-coated with a special anti-Deoxynivalenol antibody.

To carry out the assay, Deoxynivalenol (DON) standards and sample extracts respectively are pipetted into the cavities. After 15 min incubation the Deoxynivalenol peroxidase conjugate (DON-HRP-conjugate) is added without a preceding washing step.

Deoxynivalenol from the standard or sample competes with the HRP labelled Deoxynivalenol for the antibody binding sites. The unbound components are removed by rinsing them off and then peroxidase substrate solution (3,3',5,5'-Tetramethylbenzidine, TMB) is pipetted into all cavities. The DON-HRP conjugate that is bound to the antibody reacts with the substrate solution by forming a blue colour. After 15 minutes, this reaction is terminated by adding the stop solution. The colour then changes from blue to yellow, which is detected photometrically. With the help of a microplate reader, the yellow colouring is measured as an optical density (OD) at a wavelength of 450nm (reference value 620nm).

The Deoxynivalenol concentration is inversely proportional to the colour intensity. The higher the OD measurement, the lower is the concentration of Deoxynivalenol in the standard or sample.



Kit components

No.	Components	Code	Amount	Colour of the top cap	Condition *
01	Microplate; coated with anti-Deoxynivalenol-antibody	DON-PLATE	12 Strips á 8 well	-	G
02	Sample dilution buffer	DON-SAMPLE-BUF	2 x 30 mL	white 	G
03	DON-Standard 0 (0 ng/mL)	DON-0	1 x 1.0 mL	red 	G
04	DON-Standard 1 (3.07 ng/mL)	DON-1	1 x 1.0 mL	red 	G
05	DON-Standard 2 (7.68 ng/mL)	DON-2	1 x 1.0 mL	red 	G
06	DON-Standard 3 (19.2 ng/mL)	DON-3	1 x 1.0 mL	red 	G
07	DON-Standard 4 (48.0 ng/mL)	DON-4	1 x 1.0 mL	red 	G
08	DON-Standard 5 (120.0 ng/mL)	DON-5	1 x 1.0 mL	red 	G
09	DON-HRP-Conjugate	DON-HRP	1 x 6.0 mL	green 	G
10	Substrate solution (TMB)	TMB	1 x 12.0 mL	brown 	G
11	Stop solution (1M H ₂ SO ₄)	STOP-H ₂ SO ₄	1 x 4.0 mL	white 	G
12	Wash solution, 10-fold concentrate	WASH-10x	1 x 30.0 mL	white 	R
13	Adhesive foil for microplate	-	1 piece	-	G
14	Instructions	-	1 piece	-	-

* Condition: G = ready for use; R = reconstitution required

Table 1: Kit components

Additional equipment, not included in delivery

Micropipettes with their corresponding tips (range of 10-100 µL, 100-1000 µL)

Dispenser (50µl volume)

Mortar or laboratory mill

Laboratory scales

Erlenmeyer flask or plastic centrifuge tube

100mL measuring cylinder

Funnel

Stand

Filter (e.g. Whatman No. 1)

Collection containers for sample extracts

Containers for diluted sample extracts, e.g. 1mL volume

Centrifuge

Microplate shaker

Microplate washer or multi-channel pipette for washing

Microplate reader (450 nm / 620 nm)

Distilled water



Storage and shelf life

The Deoxynivalenol ELISA Assay Kit has to be stored at 2-8°C (Do not freeze!). After the expiry date has passed, the quality of the kits can no longer be guaranteed.

With a lower number of samples, individual microplate strips can also be used. The remaining strips must be put back in the foil bag with desiccant and securely closed for further storage.

Opened reagents must be used within 6 weeks and stored at 2-8°C in the meantime.

The substrate solution can no longer be used if the normally colourless solution shows a markedly blue colour. Another indication of the deterioration of the reagents would be very little or no staining at all in the cavities of maximum binding (DON-Standard 0).

Special health information

The Deoxynivalenol ELISA Assay Kit contains the toxin Deoxynivalenol. It is present as a solution in the concentration range of 3.07 – 120 ng/mL in the DON-Standards (see kit components). Contact with skin must be avoided. Therefore protective gloves and laboratory suits are recommended. Solutions and materials containing DON must be disposed of or cleaned professionally.

The stop solution (see kit components, STOP-H₂SO₄) contains 1M of sulphuric acid. Avoid contact with skin and mucus membranes. If you come into contact with it, wash it off with plenty of water. Protective gloves must be worn!

General information for the test procedure

- Kit Components from different lots should not be mixed.
- The reagents should be warmed to room temperature (20-25°C) before beginning the test.
- Neither the reagents nor the microplate may be exposed to direct sunlight.
- The substrate solution (TMB) must always be kept in the dark.
- All reagents have to be mixed carefully before use. Avoid foaming!
- Components that are not delivered ready for use must be freshly prepared before use. This concerns the washing solution that is included in the kit as a 10-fold concentrate (WASH-10x).
- Samples should always be tested at the same time as the standards. The standards should be included in each test run to check the quality of the results.
- The interior of the cavities should not be touched while pipetting to ensure the coating is not damaged.
- To avoid cross-contamination, a new pipette tip must be used for each sample. The tips should be pre-saturated with the solution to be pipetted.
- Close the kit reagent containers immediately after use. Screw caps should not be mixed up.
- All cavities of the microplate strips should be uniformly washed at the same time before the colour reaction. The washing process is critical. Insufficient washing can lead to inaccurate results.
- Do not allow the microplate to dry after washing.
- The test should be carried out as described in the manufacturer's instructions. The specified times must be adhered to. Work should be carried out quickly.
- Check the precision and accuracy of the laboratory equipment that you use during the process.



Sample preparation

Solid samples (cereals, feed)

- Solid samples should be present in crushed, homogeneous form as a fine to medium-fine powder. If necessary, the solid sample must be crushed in a mortar or with a laboratory mill.
- 5g of the crushed, homogeneous sample is weighed in a suitable container, such as an Erlenmeyer flask or a plastic centrifuge tube and mixed with 25mL of water. This suspension is shaken intensively for 3 minutes to extract the Deoxynivalenol. Centrifuge the extract at 3000 g for 5 min. The extract is then filtered via a folded filter for quantitative analysis.
- The filtrate (sample extract) is diluted in a new container with a 1:5 ratio with the sample diluent (see kit components, DON-SAMPLE-BUF):

1 part filtrate + 4 parts DON-SAMPLE-BUF, e.g. 200 µL filtrate + 800 µL DON-SAMPLE-BUF
- The diluted filtrate can now be tested with the immunoassay. 1 mL of diluted filtrate equals 0.04 g solid sample = **dilution factor 25**.

Beer

- Carbonized beer samples should be degassed by moderate heating.
 - Dilute 100 µL beer sample with 900 µL DON-SAMPLE-BUF
 - The diluted sample can now be tested with the immunoassay. 1 mL of diluted filtrate equals 0.1 mL beer sample = **dilution factor 10**
- **Note:** If higher Deoxynivalenol concentrations are expected, then the sample must be diluted at a higher ratio than 1:25 (solid samples) or 1:10 (beer). The ratio of the solid sample/water mixture (1/5, g/v) must be maintained.

Test procedure

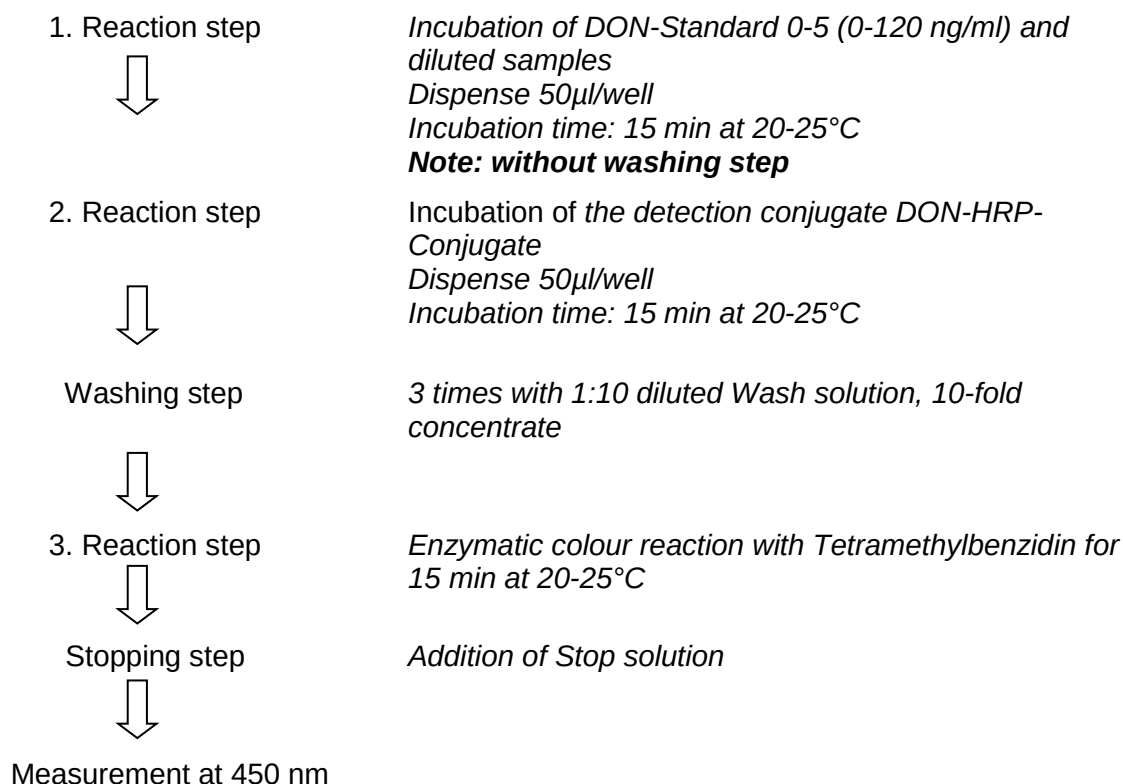
Note: It is recommended to test both the standards and the samples in duplicate and further, that no more than 18 samples and 6 standards (maximum of 6 strips) are measured in one run.

- The microplate (DON-PLATE) is already ready for use; it must only be taken from the foil bag and can be used immediately without washing.
- The washing solution comes in a 10-fold concentration (WASH-10x) and must be diluted with distilled or deionised water: fill 25mL WASH-10x with 225mL of water at a volume of 250mL.
- 50 µL of the standards (DON-Standard 0 to DON-Standard 5) and the prepared sample extract are pipetted into the corresponding cavities. The microplate is now covered with adhesive foil and briefly shaken on the microplate shaker and incubated for 15 minutes at room temperature, protected from light.
- 50µl DON-HRP conjugate (DON-HRP) is pipetted into all cavities without preceding washing step.
The microplate is now covered with adhesive foil again and briefly shaken on the microplate shaker and incubated for 15 minutes at room temperature, protected from light.
- All cavities are aspirated or shaken out for washing. Then 300 µL of reconstituted washing solution is pipetted into each cavity and aspirated out again. This process is repeated twice.
- 100 µL of substrate solution (TMB) is then pipetted into all cavities for the colour reaction.



- Cover the microplate with adhesive foil again, incubate it with shaking for 15 minutes at room temperature, protected from light.
- To stop the process, 25 µL of stop solution (STOP-H₂SO₄) is pipetted into each cavity.
- The OD value measurement of the cavities is undertaken by using a microplate reader at 450nm (reference wave length 620nm).

Reaction scheme



Test evaluation

- First, the mean values of all duplicate results for the optical density (O.D. 450nm) are calculated.
- The results can be evaluated easily and quickly by creating a standard curve. For this, the mean optical density of the standards is plotted against their concentration [ng/mL] semi-logarithmic. (x-axis: log Deoxynivalenol [ng/mL]; y-axis: mean optical density).
- Using the mean optical density of the sample, the corresponding concentration of Deoxynivalenol [ng/mL] can be determined in a measured, diluted sample extract from this standard curve. The content of the sample is calculated in consideration of the factors for the sample dilution. When using the above-described sample preparation, the dilution factor is 25 for solid samples or 10 for beer samples. Thus the Deoxynivalenol concentration from the sample is obtained in ppb [ng/g or µg/kg].
- A Logit/log evaluation is also an option. For this procedure, the values for the mean optical density of the standards and samples are divided by the mean value of the zero standard (DON-0). Multiplying by 100 then gives an O.D. value percentage in reference to the zero standard (100%):

$$\frac{\text{O.D. (Standard or Sample)}}{\text{O.D. Standard 0}} \times 100 = \% \text{ O.D.}$$



The Logit/log evaluation takes place by using the O.D. value percentage as well as the logarithmic concentration value. This returns a linear standard curve. Implementing a linear regression is thus an option.

- Is it possible to perform a non-linear regression, the following function is proposed:
$$Y = pr1/(pr2+X1)+pr3.$$

Note: For each evaluation is valid: Multiply the concentration value by dilution factors to obtain the effective DON concentration in the original test sample.

Example of standard value and standard curve

DON [ng/ml]	Mean O.D.	CV [%]	% O.D.
0	1.445	2.6	100.0
3.07	1.127	3.6	78.0
7.68	0.891	4.3	61.7
19.2	0.626	6.0	43.3
48	0.419	3.3	29.0
120	0.271	6.6	18.8

Table 2: Example of standard value with DON-Standard 0-5

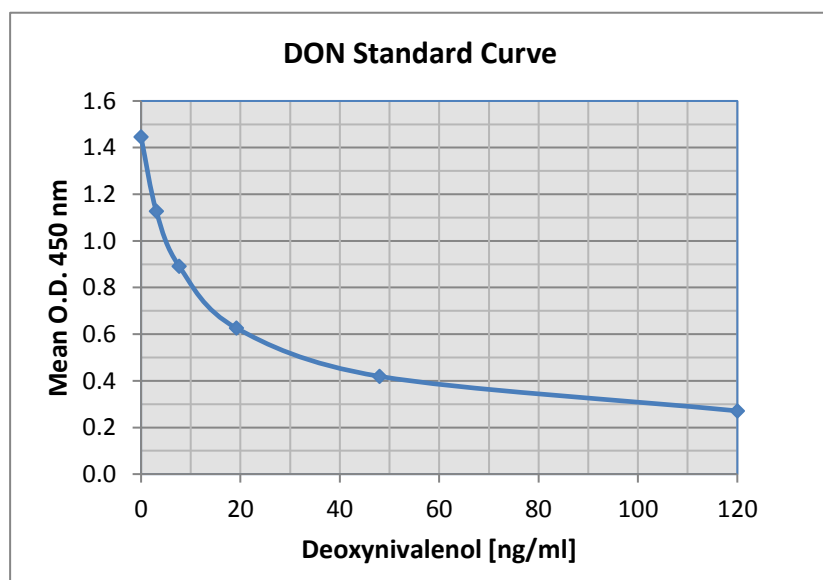


Fig. 1: Example of standard curve with DON-Standard 0-5

Note: The standards must be run with each test.



Performance features of the method

Accuracy

The limit of detection (LOD) is defined as the mean plus two standard deviations of multiple determinations of a DON free sample extract. Different commodities can have an influence on the LOD due to their "matrix effects".

As a result, the LOD may be dependent on the matrix and should be measured for each different commodity.

The LOD of the Deoxynivalenol ELISA Assay Kit for blank samples of 2 commodities was estimated in 10 measurements.

LOD wheat flour: 12 ppb

LOD oats: 15 ppb

Recovery

The recovery of spiked samples was found to be 80% to 110%

Linearity

The assay should be carried out with a filtrate dilution of 1:25 for solid samples or 1:10 for beer samples. With dilutions of 1:30 to 1:50 for solid samples, the linearity of the assay results is given as their dilution. With dilutions of 1:20 to 1:40 for beer samples, the linearity of the assay results is given as their dilution.

Intra assay accuracy

The intra assay variation of the Deoxynivalenol test has been determined as $\leq 6\%$ (n=16).

Inter assay accuracy

The inter assay variation of the Deoxynivalenol test has been determined as $\leq 12\%$ (n=10).

Specificity

Specificity studies have shown the following cross-reactivity:

Deoxynivalenol:	100 %
15-Acetyldeoxynivalenol:	>100 %
3-Acetyldeoxynivalenol:	<1 %
T2-Toxin:	<1 %
Nivalenol:	<1 %

Table 3: cross-reactivity





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