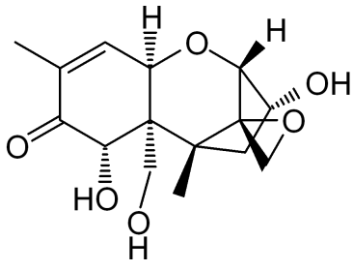
SENSI*Spec* ELISA Deoxynivalenol (DON) 96 Tests

Enzyme Immunoassay for the Quantitative Determination of Deoxynivalenol in Food (Cat.nr. HU0030084)

Sensitivity	0.5 ng/mL
Recovery (spiked samples)	90-110%
Incubation Time	60 min

1. GENERAL INFORMATION



Deoxynivalenol (DON, Vomitoxin) in addition to zearalenone, the fumonisines and other trichothecenes belongs to the fusarium toxins. These toxins are already produced on the field in consequence of a contact of the cereals by fusarium species. Acute toxic dosages result in sickness and emesis. Deoxynivalenol is a gastrointestinal irritant and an inhibitor in protein synthesis. Farm animals react with a delay of growth and a depressed immune system resulting in a higher sensitivity for infections.
Since July 1st, 2006 the following maximum amounts of deoxynivalenol are valid throughout the EU:

Raw cereals	1250-1750 ppb
Flour	750 ppb
Bakery products	500 ppb
Baking ingredients (dry)	750 ppb
Baby food	200 ppb

Since June 2010 the FDA recommends maximum amounts of 1000 ppb for cereal products and 10000 ppb for raw cereals. Thus an observation of food and feed with respect to the concentration of deoxynivalenol is obligatory.
The **SENSISpec Deoxynivalenol ELISA** represents a highly sensitive detection system and is particulary capable of the quantification of deoxynivalenol contaminations in cereals, beer and soy.

2. PRINCIPLE OF THE TEST

The **SENSISpec Deoxynivalenol** quantitative test is based on the principle of the enzyme-linked immunosorbent assay. An antibody binding protein is coated on the surface of a microtiter plate. Deoxynivalenol containing samples or standards and an antibody directed against deoxynivalenol are given into the wells of the microtiter plate. The deoxynivalenol contained in samples or standards will bind to the antibody which reacts with the binding protein coated onto the microtiter plate. After 30 minutes incubation at room temperature a deoxynivalenol-peroxidase conjugate is added into the wells without a preceding washing step to saturate free antibody binding sites. After additional 15 minutes incubation at room temperature the wells are washed with diluted washing solution to remove unbound material. A substrate solution is added and incubated for 15 minutes, resulting in the development of a blue colour. The colour development is inhibited by the addition of a stop solution, and the colour turns yellow. The yellow colour is measured photometrically at 450 nm. The concentration of deoxynivalenol is indirectly proportional to the colour intensity of the test sample.

3. PRECAUTIONS

Full compliance of the following good laboratory practices (GLP) will determine the reliability of the results:

1) Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).

2) All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.

3) Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.

4) Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.

5) Use a separate disposable tip for each specimen to prevent cross-contamination.

6) All specimens and standards should be run at the same time, so that all conditions of testing are the same.

7) Do not mix components from different batches.

8) Do not use reagents after expiration date.

9) Check both precision and accuracy of the laboratory equipment used during the procedure (micropipets, ELISA reader etc.).

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4. HEALTH AND SAFETY INSTRUCTIONS

1) Do not smoke or eat or drink or pipet by mouth in the laboratory.

2) Wear disposable gloves whenever handling patient specimens.

3) Avoid contact of substrate and stop solution with skin and mucosa (possible irritation, burn or toxicity hazard). In case of contact, rinse the affected zone with plenty of water.

4) Handling and disposal of chemical products must be done according to good laboratory practices (GLP).

5. REAGENTS

The kit contains reagents for 96 determinations. They have to be stored at 2-8°C. Expiry data are found on the labels of the bottles and the outer package.

1) Microtiter plate consisting of 12 strips with 8 breakable wells each, coated with anti-mouse antibody.

2) Deoxynivalenol Standards (0, 2, 8, 20, 40, 80 ng/mL): 6 vials with 1 mL each, dyed red, ready-to-use.

3) Anti-Deoxynivalenol Antibody (mouse): 6 mL, dyed red, ready-to-use.

4) Conjugate (Deoxynivalenol-Peroxidase): 6 mL, dyed red, ready-to-use.

5) Substrate Solution (TMB): 15 mL, prestained red, ready-to-use.

6) Stop Solution (0.5 M H_2SO_4): 15 mL, ready-to-use.

7) Sample Diluent (PBS): 2 x 60 mL, dyed red, ready-to-use.

8) Washing Solution (PBS + Tween 20): 60 mL as 10x concentrate. Dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.

9) Two plastic foils to cover the strips during the incubation.

10) Plastic bag to store unused microtiter strips.

11) Instruction Manual.

6. ADDITIONAL INSTRUMENTATION AND REAGENTS (not provided)

Instrumentation

- 50, 100, 500 and 1000 µL-micropipets
- ELISA reader (450 nm)
- Centrifuge
- Ultra-Turrax, mixer, vortex

Reagents

- Double distilled water
- Potassiumhexacyanoferrate(II)-3-hydrate (150 g/L; Carrez I)
- Zincsulfate-7-hydrate (300 g/L; Carrez II)

7. SAMPLE PREPARATION

Cereals

- Suspend 4 g of previously ground sample in 20 mL of double distilled water.
- Mix suspension for 5 minutes.
- Centrifuge extract at 3000 g for 10 minutes.
- Dilute 200 µL of supernatant with 800 µL of sample diluent and test the sample in the ELISA.
- Sample dilution factor: F=25.

Beer / Gyle

- Carbonized beer samples should be previously degassed by moderate heating.
- Cloudy beers (such as beer brewed from wheat) / gyle should previously be sterile-filtered.
- Dilute 100 µL beer / gyle with 900 µL sample diluent.
- Sample dilution factor: F=10.

Soy

- Suspend 4 g of previously ground sample in 20 mL of double distilled water.
- Mix suspension for 5 minutes.
- Centrifuge extract at 3000 g for 10 minutes.
- Add 100 µL Carrez I to 1 mL supernatant, mix well and add 100 µL Carrez II afterwards.
- Mix sample and centrifuge at 3000 g for 10 minutes.
- Dilute 250 µL of supernatant with 800 µL of sample diluent and test the sample in the ELISA.
- Sample dilution factor: F=25.

8. PROCEDURE

1) Prepare samples as described above.

2) Pipet 100 µL standards or prepared samples in duplicate into the appropriate wells of the microtiter plate. Immediately add 50 µL anti-deoxynivalenol antibody into each well.

3) Cover the microtiter plate with a plastic foil and incubate for 30 minutes at room temperature.

4) Without preceding washing add 50 µL deoxynivalenol-peroxidase conjugate into each well.

5) Cover the microtiter plate with a plastic foil and incubate additional 15 minutes at room temperature.

6) Wash the plate three times as follows: Discard the contents of the wells (dump or aspirate). Pipet 300 µL of diluted washing solution into each well. After the third repetition empty the wells again and remove residual liquid by striking the plate against a paper towel. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbencies.

7) Pipet 100 µL of substrate solution into each well.

8) Allow the reaction to develop in the dark (e.g. cupboard or drawer; the chromogen is light-sensitive) for 15 minutes at room temperature.

9) Stop enzyme reaction by adding 100 µL of stop solution (0.5 M H_2SO_4) into each well. The blue colour will turn yellow upon addition.

10) After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The colour is stable for 30 minutes.

9. CALCULATION OF RESULTS

1) Calculate the average optical density (OD 450 nm) for each set of reference standards or samples.

2) Construct a standard curve by plotting the mean optical density obtained for each reference standard against its concentration in ng/mL on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis.

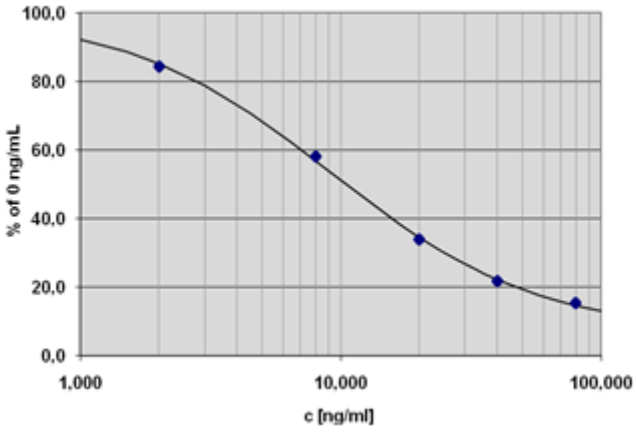
3) Using the mean optical density value for each sample, determine the corresponding concentration of deoxynivalenol in ng/mL from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.

4) The diluted samples must be further converted by the appropriate **sample dilution factor**. The factors are listed for each sample matrix in the *sample preparation* section.

10. TYPICAL STANDARD VALUES

The following table contains an example for a typical standard curve. The binding is calculated as percent of the absorption of the 0 ng/mL standard. These values are only an example and should not be used instead of the standard curve which has to be measured in each new test.

Deoxynivalenol (ng/mL)	(% binding of 0 ng/mL)
0	100
2	84
8	58
20	34
40	22
80	15



11. PERFORMANCE

Sensitivity

The limit of detection (LOD) of the **SENSISpec Deoxynivalenol test** is 0.5 ng/mL.

The limit of quantification (LOQ) of the **SENSISpec Deoxynivalenol test** is 2 ng/mL.

Due to the variety of sample matrices and their influence on the blank, results less than the LOQ should be treated as negative.

11.1. Cross-reactivity

Cross-reactivity relative to deoxynivalenol (=100%)

15-acetyl-Deoxynivalenol	0.4%
3-acetyl-Deoxynivalenol	800%
Deoxynivalenol 3-glucoside	102%

11.2. Intra-assay Precision

The intra-assay variation of the deoxynivalenol test was determined to 5%.

11.3. Recovery

Mean recovery was determined by spiking samples with different amounts of sesame:

Soup	100%
Ice	85%
Sausage	92%
Salad sauce	93%
Cracker	109%

12. REFERENCES

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