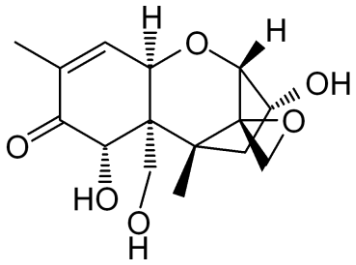
SENSI*Spec* ELISA Deoxynivalenol (DON) RAPID 96Tests

Enzyme Immunoassay for the Rapid Quantitative Determination of Deoxynivalenol in Cereals and Beer /Gyle (Cat.nr. HU0030081)

Sensitivity	0.04 – 0.16 ppm
Recovery (spiked samples)	97 - 107%
Incubation Time	20 min

1. GENERAL INFORMATION



Deoxynivalenol (DON, Vomitoxin) in addition to zearalenone, 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 June 2010 the US Food and Drug Association recommends maximum amounts of 1000 ppb for finished cereal products and 2000 - 10000 ppb for raw cereals depending on the intended use. Thus an monitoring of food and feed with respect to the concentration of deoxynivalenol is obligatory.

The **SENSISpec Deoxynivalenol RAPID ELISA** represents a highly sensitive detection system and is particulary capable of the rapid quantification of deoxynivalenol contaminations in cereals and beer.

2. PRINCIPLE OF THE TEST

The **SENSISpec Deoxynivalenol RAPID** quantitative test is based on the principle of the enzyme-linked immunosorbent assay. A microtiter plate is coated with antibodies raised against mouse immunglobulins. Deoxynivalenol containing samples or standards, a deoxynivalenol-peroxidase conjugate and an antibody directed against deoxynivalenol are given into the wells of the microtiter plate. The conjugate competes with the deoxynivalenol of samples/standards for the limited number of antibody sites. Simultaneously the anti-deoxynivalenol antibody is bound to the anti-mouse antibody coated on the microtiter plate. After 10 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 10 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.).

4. HEALTH AND SAFETY INSTRUCTIONS

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

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2) 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.

3) 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, 0.2, 0.5, 1, 2, 5 ppm): 6 vials with 1 mL each, dyed red, ready-to-use. Because of the total dilution of 1:25 of the cereal samples in the extraction step, the calibrators contain 1/25th of the stated value. Thus no further calculation after analysis is necessary.

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

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

5) Substrate Solution (TMB): 15 mL, 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) Plastic bag to store unused microtiter strips.

10) Instruction Manual.

6. ADDITIONAL INSTRUMENTATION AND REAGENTS (not provided)

Instrumentation

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

Reagents

- double distilled water

7. SAMPLE PREPARATION

Cereals

- Grind sample to pass through a 20 mesh sieve and thoughly mix prior to sub-sampling.
- Suspend 20 g of sample in 100 mL of double distilled water.
- Mix suspension for 5 minutes.
- Filter through Whatman #1 filter or alternatively centrifuge at a minimum of 3000 g for 5 minutes.
- Dilute 100 µL of filtrate/supernatant with 400 µL of sample diluent and test the sample in the ELISA.

Beer / Gyle

- Carbonized beer samples should be preliminarily degassed by moderate heating.
- Cloudy beers (such as beer brewed from wheat) / gyle should preliminarily be sterile-filtered.
- Dilute 100 µL beer / gyle with 900 µL sample diluent.

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.

3) Add 50 µL of deoxynivalenol-peroxidase conjugate into each well.

4) Add 50 µL of the anti-dexoynivalenol antibody into each well.

5) Incubate for 10 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 10 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

The ready-to-use standards are prepared for a direct determination of cereal sample concentrations. The dilution of samples in the extraction process as described in the above stated sample preparation procedure is already considered. Additional dilution due to high sample concentration has to be accounted for.

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 ppm on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis. Alternatively the evaluation can be carried out by software. In this case the 4-parameter method should be preferred.

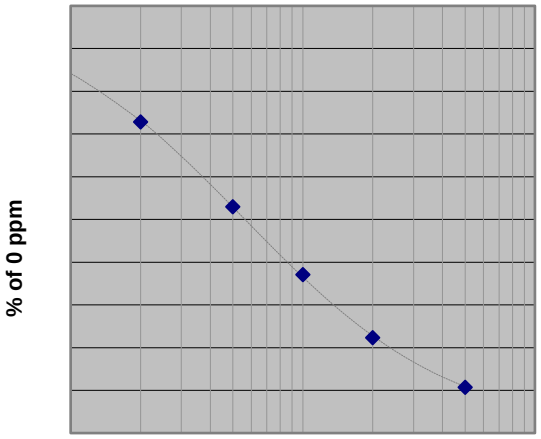
3) Using the mean optical density (OD) value for each sample, determine the corresponding concentration of deoxynivalenol in ppm from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.

4) Due to a deviating sample preparation process the results for **Beer / Gyle samples additionally have to be multiplied with 0.4** in order to get the real concentration of the sample.

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 ppm 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 (ppm)	(% binding of 0 ppm)
0	100
0.2	73
0.5	53
1	37
2	22
5	11



11. PERFORMANCE

Sensitivity

The limit of detection (LOD) of the **Deoxynivalenol RAPID test** is 0.08 ppm.

Validation experiments with common matrices resulted in the following LODs [ppm].

Wheat	0.11
Rye	0.15
Barley	0.11
Oats	0.15
Corn	0.16
Rice	0.15
Beer	0.04

The limit of quantification (LOQ) of the **Deoxynivalenol RAPID test** is 0.2 ppm.

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	50%

11.2. Precision

Intra-assay Precision	3-5%
Inter-assay Precision	7-10%

11.3. Linearity

The serial dilution of spiked samples (wheat, oats, rice, corn and beer) resulted in a dilution linearity of 81-104%.

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11.4. Recovery

Wheat flour	97%
Oats flour	98%
Rice flour	107%
Corn flour	102%
Beer	104%

12. REFERENCES

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