Deoxynivalenol (Rapid/HS) ELISA Kit

Enzyme Immunoassay for the rapid quantitative determination of Deoxynivalenol in cereals and beer/gyle

Catalog number: ARG80795

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INTRODUCTION

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	1.250-1.750 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.

PRINCIPLE OF THE ASSAY

This assay employs the competetive quantitative enzyme immunoassay technique. An antibody is coated on the surface of a microtiter plate. 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 TMB solution is add incubated for 10 minutes, resulting in the development of a blue color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The yellow color is measured at 450 nm. The concentration of deoxynivalenol is indirectly proportional to the color intensity of the test sample.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8-well	4°C
Anti-Deoxynivalenol Antibody	6 ml (ready to use)	4°C
HRP-Deoxynivalenol Conjugate	6 ml (ready to use)	4°C
Standard A-F (0, 0.2, 0.5, 1, 2, 5 ppm)	6 X 1 ml (ready to use)	4°C
Sample dilution buffer	2 X 60 ml	4°C
10x Wash Buffer	60 ml	4°C
TMB substrate	15 ml	4°C (Protect from light)
STOP solution	15 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
- Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
- If crystals are observed in the 10X Wash buffer, warm to RT or 37°C for 15 min or until the crystals are completely dissolved.
- All specimens and standards should be run at the same time, so that all conditions of testing are the same.

- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

Cereals

- Grind sample to pass through a 20 mesh sieve and 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 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.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 10X wash buffer into distilled water to yield 1X wash buffer. (E.g. 50 ml of 10X Wash buffer + 450 ml of distilled water) Store the diluted Wash buffer at 2°C to 8°C.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µl** of **standards and samples** in duplicate into wells.
3. Add **50 µl** of **deoxynivalenol-peroxidase conjugat**e into each well.
4. Add **50 µl** of the **anti-dexoynivalenol antibody** into each well.
5. Incubate for **10 minutes at RT.**
6. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes.** Wash by filling each well with **1X wash buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
7. Add **100 µl** of **TMB substrate mixture** to each well. Incubate for **10 minutes at room temperature** in dark.
8. Add **100 µl** of **Stop Solution** to each well.
9. Read the OD with a microplate reader at **450 nm immediately** (optional:

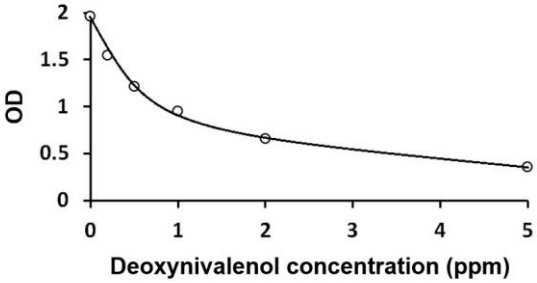
read at 620 nm as the reference wave length) It is recommended read the absorbance within 30 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. 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 for data from cereal samples. Due to a deviating sample preparation process the results for Beer / Gyle samples additionally data from Beer / Gyle samples have to be multiplied with 0.4 (10/25, Beer / Gyle dilution factor =10, and the standard has been diluted 25X) in order to get the real concentration of the sample.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The limit of detection (LOD) of the Deoxynivalenol ELISA kit is 0.08 pom.

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 ELISA kit is 0.2 ppm.

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

Specificity

For the following foods no cross-reactivity could be detected:

Cross-reactivity	Relative to deoxynivalenol (=100%)
15-acetyl-Deoxynivalenol	0.4%
3-acetyl-Deoxynivalenol	800%
Deoxynivalenol 3-glucoside	50%

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 3-5% and Inter-assay precision was 7-10%.

Recovery

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