Soft Flow

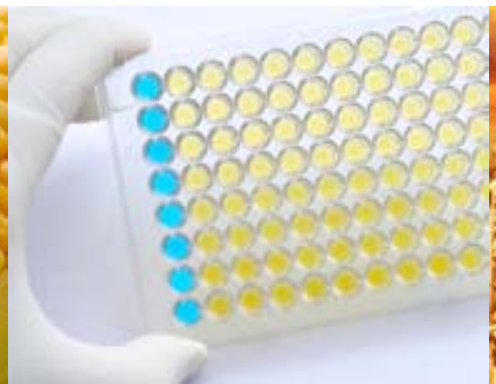
TOXI-WATCH

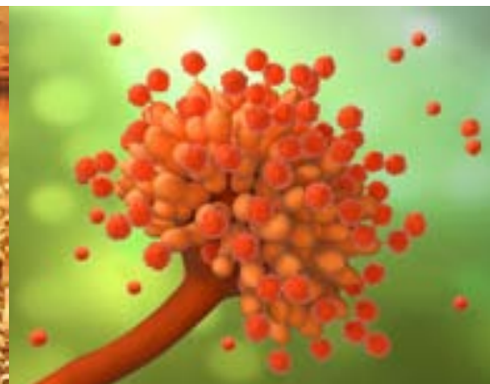
TECHNICAL DATA SHEET

DEOXYNIVALENOL ELISA Kit

a competitive immunoassay for the quantitative analysis of mycotoxins, occurring in feed

A3000031



BRIEF PRODUCT INFORMATION

The Kit is developed for the quantitative analysis of Deoxynivalenol occurred mainly in wheat and corn suggested for animal feeding.

This product is for in vitro investigation/research use only (RUO). Not intended for clinical or diagnostic use.

The TOXI-WATCH DEOXYNIVALENOL ELISA Kit is a competitive, enzyme-linked immunosorbent assay (ELISA) method for the quantitative analysis of the Deoxynivalenol (hereafter DON) mycotoxin occurring in some cereals and feed.

The provided materials (in Kit, described below) are sufficient for the (simultaneous) analysis of (even) 96 samples (including the standards).

A microplate/ELISA reader is necessary for the measurement, but no special training or equipment is required for an experienced laboratory technician to perform the test.

Lot Number:	
Expiration:	
Catalogue number:	A3000031
Duration of test:	Extraction: 15 min. Test: 45 (incubation) + 15 min.
Range of test:	625 – 20.000 ppb (applied standard range: 1.25 – 40 ng/mL)
Sensitivity of test:	370.5 ppb
Storage and Stability:	Store the kit at 2 – 8 °C (35 – 46 °F). Do not freeze any test kit components. *If the absorbance of the 0 standard (CAL0) is less than 0.7 or blue coloration of the substrate is observed, do not use and discard the test!*

TABLE OF CONTENTS

COMPONENTS OF KIT

- A Wash buffer (**WASH**, 10x concentrated, 15 mL, 1 pc.)
- B Stop solution (**STOP**, ready to use, 8 mL, 1 pc.)
- C DON-HRP conjugate (**CONJ**, 50× concentrated, 0.4 mL, 1 pc.)
- D Substrate solution (**SUB**, ready to use, 25 mL, 1 pc.)
- E Streptavidin coated microtiter plate, 12 × 8 separable strips (**MP**, 1 pc.)
- F Anti-Deoxynivalenol antibody (**AS**, biotinylated, ready to use, 6 mL, 1 pc.)
- G Calibration solutions (**CAL 1-6**, ready to use, 6 × 0.5 mL, 6 pcs.)
  - ***CAL 0*** *= PBS buffer (0.01M, pH=7.4), not provided, prepared by user*
  - ***CAL 1*** *= 1.25 ng/mL, 0.5 mL (ready to use, 1 pc.)*
  - ***CAL 2*** *= 2.5 ng/mL, 0.5 mL (ready to use, 1 pc.)*
  - ***CAL 3*** *= 5 ng/mL, 0.5 mL (ready to use, 1 pc.)*
  - ***CAL 4*** *= 10 ng/mL, 0.5 mL (ready to use, 1 pc.)*
  - ***CAL 5*** *= 20 ng/mL, 0.5 mL (ready to use, 1 pc.)*
  - ***CAL 6*** *= 40 ng/mL, 0.5 mL (ready to use, 1 pc.)*
- H Extraction buffer (**SDB**, 10x concentrated, 230 mL, 1 pcs, in 1 bottle.)

PRINCIPLE OF THE TEST

The method of the Kit is based on the antigen–antibody binding reaction. Wells of a microtiter plate are coated with Streptavidine, on to which the toxin standards, samples and/or the **DON**-horseradish peroxidase (**HRP**) conjugate (as antigens) are pipetted.

These components compete for the antigen-binding sites of the biotinylated antibody highly specific to DON (competitive ELISA). Contemporarily, the antibody is intensely bound on the surface of the wells. The unbound **DON-HRP** conjugates (and other antigens) are eliminated/removed with the following wash procedure (use **WASH** buffer).

The colorless substrate turns blue in proportion with the quantity of the bound **DON-HRP** conjugate. The reaction is stopped; the color changes from blue to yellow. Color intensity, optical density (OD) can be measured with a microplate reader at 450 nm (and a differential filter of 630 nm). The measured absorbance is inversely proportional to the concentration of Deoxynivalenol of samples.

PRECAUTIONS

This test should only be carried out by trained laboratory employees. The instruction for use must be strictly followed.

The calibration solutions (**CAL1-6**) and the conjugate (**CONJ**) contain mycotoxins that may have adverse, carcinogen, and/or mutagen effects in humans and animals. Use latex gloves and safety glasses when handling the toxins.

Decontamination of the glassware and toxin-content solutions is best carried out using a sodium hypochlorite (bleach) solution (10 %; v/v) overnight (adjust solution with HCl to pH=7.0).

The **STOP** solution contains 1N sulfuric acid (R36/38, S2-26). Wash the affected area with plenty of water if spilled on skin. Refer to the MSDS for more information.

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

Equipments:

- grain mill
- plate/orbital shaker (max. 300 rpm)
- Erlenmeyer flasks (125 mL)
- graduated cylinder (minimum 100 mL) and aluminium foil
- vortex
- microplate (ELISA) reader (equipped with 450 nm filter)
- micropipettes and suitable/fitting tips
  - 1 channel (20 - 200 µL, 200 - 1000 µL, suggested use for pipetting samples and the standard solution)
  - 8 channel (20 - 200 µL, suggested use for the pipetting of **CONJ**, **AS**, **SUB**, **STOP**)
- test tubes or centrifuge tubes (*e.g.* Eppendorf tubes)
- glass funnel, filter paper (with 4 - 7 µm suggested pore size)
- microplate washer (optional)
- balance
- computer with data evaluation software (optional)
- latex gloves

Materials:

- Distilled/ultra-filtered water
- Phosphate buffered saline (PBS, 0.01 M, pH = 7.4)
- 10 % sodium-hypochlorite solution (bleach)
- *CAL 0 = PBS buffer (0.01M, pH=7.4), not provided, it should be prepared by user*

Extraction procedure:

- Remove your cereals/feed samples from storage (see useful tips!). Prepare finely ground cereal samples, homogenize.
- Prepare 1 x CC. SDB Buffer (an example: dilute 50 mL 10 × CC. SDB (**SDB**) with 450 mL distilled water).
- Add 5 grams of the sample into Erlenmeyer-flask, add 25 mL extraction buffer (freshly prepared 1 x CC. SDB, see above!) and shake for 15 minutes (at 300 rpm). Wait for 5 minutes (for the sufficient sedimentation).
- Filter the extracts through filter paper and collect them in test tubes or centrifuge tubes.
- Dilute the extracts with PBS buffer 100 times (*e.g.* 990 µL PBS buffer + 10 µL extract).

DESCRIPTION OF THE TEST

USEFUL TIPS

- Let the microtiter plate (**MP**) warm up to RT before opening the package. Take out as many wells, as needed. The remaining strips should be kept in the original foil bag at 4°C.
- Let all reagents warm up to RT, and shake them well before use (except the antibody, which should be shaked gently). However, take out (into test tubes) only the needed amount of **AS**, **CONJ** compounds and put back those components to refrigerator. Warning: The rest of compounds should be returned to the refrigerator as soon as possible to avoid unwanted deterioration!
- Prepare all reagents for the next step in advance so when the incubation time expires, you can start the next step without any delay.
- The cereal/feed samples should be stored in a cool place, protected against light.
- An 8-channel pipette is recommended for minimizing time shift among wells. Usage of different reservoirs and tips for different components are also suggested.
- Monitor color development after the substrate has been added. Stop the reaction before the 10 min. incubation expires if strong color development occurs. On the contrary, let color developments go on further, if weak development occurs. Some experience may be needed to correctly judge the color development.
- Some crystallization may occur in **WASH** and **STOP** at 4 °C. The crystals should dissolve at RT.
- Do not use expired Kit or its components for the measurements.
- Do not interchange individiual reagents between kits of different lot numbers.
- Some kit components can be light-sensitive, therefore, avoid exposure to direct light.

Assay Procedure:

- Remove the kit from refrigerator at least 30 minutes before use to let the reagents equilibrate with the room temperature (RT). Do not open the plate (**MP**) while it is cold in order to avoid water condensation on the surface of the wells. See useful tips below!
- Prepare (1×) Wash Buffer by diluting 15 mL Wash Buffer (10×CC., **WASH**) with 135 mL distilled water.
- Dilute the conjugate (**CONJ**) DON-HRP to 50× with PBS buffer (*e.g.* 2450 µL PBS + 50 µL DON-HRP). Store it in a dark place (*eg.* covered with silver foil).
- Open the **MP** foil and transfer the strips that you want to save for later use into an empty frame, and put them back into the refrigerator (2 – 8 °C, 35 – 46 °F) in the original package! Wash the strips in current use with 200 µL distilled water two times. Shake the solution firmly and get the residue of the solution by tapping it gently to a clean paper towel.
- Pipette 50 µL from 0.01 M PBS buffer (sample diluent) as a 0 calibrator
- Pipette 50 µL from the DON standard solutions (**CAL 1-6**) into the appropriate wells.
- Pipette 50 µL of each filtered and diluted sample extracts into the appropriate wells.
- Pipette 50 µL diluted conjugate (**CONJ**) into each well.

DESCRIPTION OF THE TEST

PROCEDURES

- Pipette 50 µL antibody (**AS**) into each well. This step should be done relatively quickly with an 8-channel pipette. Move the plate gently by hand for adequate mixing of the solution.

- Incubate the plate (**MP**) in dark at RT for 45 minutes.

- After the incubation time elapsed, empty the plate into a reservoir containing 10 % sodium-hypochlorite solution with a firm movement and wash the wells 5 times with 200 µL (1x) Wash Buffer (**WASH**).

- Pipette 150 µL of substrate solution (**SUB**) in each well quickly with an 8-channel pipette, shake the plate by hand a bit, and incubate the plate in dark at RT for 10 minutes. Blue color will develop.

- Stop the reaction by adding 50 µL of **STOP** solution in each well relatively quickly with an 8-channel pipette. The blue color will turn to yellow.

- Read the plate (yellow color absorbance) with a microplate photometer at 450 nm within 5 minutes after stopping the reaction.

INTERPRETATION OF THE RESULTS

Plot the DON standard absorbance points (on the basis of measured OD values) on the vertical (y) axis vs. DON standard concentration in a log10 scale on horizontal (x) axis.

Apply a curve fitting on the standard points, and based on the OD parameters of the samples read the toxin concentrations. You will have the results in ng/mL. Calculate the DON-contents considering the concentrations and dilutions.

Application of paralels (samples, standards) and/or references are strongly suggested in measurements.

TYPICAL STANDARD CURVE

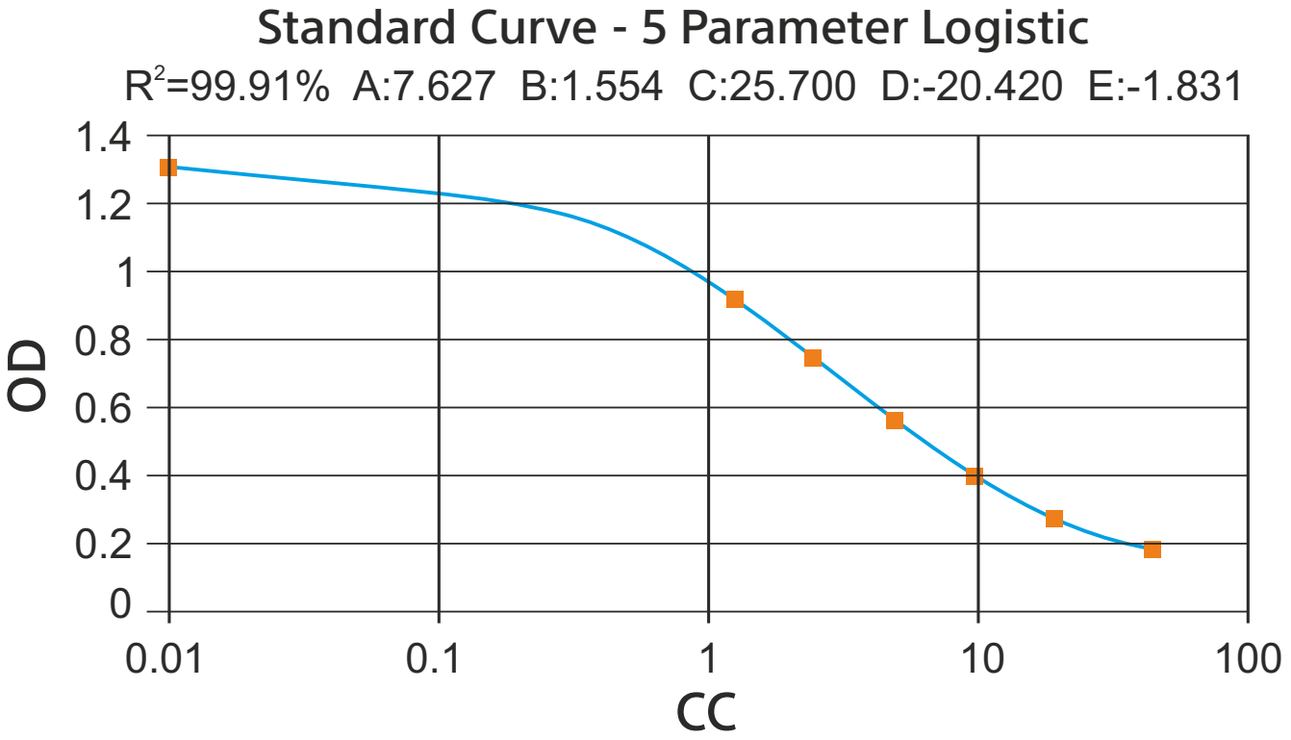


Figure 1 – Typical, Log regression standard curve using the Kit
X axis denotes the mycotoxin standard concentrations (given in ng/mL),
Y axis denotes OD values measured

IMPORTANT NOTES FOR CALCULATION

Result: toxin content of the sample in ppb:

DON content of the sample (ppb) = read DON concentration from plot (ng/mL) x 500 (dilution factor)
e.g.: if the read DON concentration is 5 ng/mL = 5 x 500 µg/kg = 2500 ppb DON

If the microplate reader software comes with an evaluation feature, use 5 or 4 parameter logarithmic or sigmoid curve fitting!

Disclaimer

The information disclosed herein is not to be construed as a recommendation to use the above product in violation of any patents. Soft Flow Hungary Ltd. will not be held responsible for patent infringement or other violations that may occur with the use of this product.

Abbreviations

DON	Deoxynivalenol mycotoxin
ELISA	Enzyme-linked immunosorbent assay
HRP	Horseradish peroxidase
CC	Concentrated
OD	Optical density
PBS	Phosphate buffered saline
ppb	Parts per billion
RT	Room temperature (20-25 °C, 68-77 °F)

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