Product information

**Background** | Deoxynivalenol (DON), called vomitoxin, is a toxic metabolite mainly produced by Fusarium graminearum. It is mainly found in wheat, barley, corn and feed. It affects immune system including embryonic toxicity and teratogenic effects. ELISA Test Kit is a competitive enzyme immunoassay for the quantitative analysis of Deoxynivalenol in cereal grains, feed and similar food samples. Deoxynivalenol ELISA Test Kit incorporates a fast, low-cost sample preparation protocol with high recovery rates.

An antibody with affinity for Deoxynivalenol is coated onto wells of ELISA plate. Standard or sample are added to an appropriate well and if Deoxynivalenol is present it will bind to the coated antibody. Subsequently, Deoxynivalenol bound to horse-radish peroxidase (HRP) is added and it binds to the antibody not already occupied by Deoxynivalenol present in the sample or standard. Following an incubation period, liquid in respective wells is decanted, washed and a HRP substrate is added to allow development of a blue color in the presence of an enzyme. The resulting color intensity, has an inverse relationship with the target concentration in an analyzed samples. The levels of Deoxynivalenol are determined by comparison of the color intensity of unknown samples with the standard curve, which is made using the Deoxynivalenol standards provided in the kit.

**Detection limit:** grain, feed – 500 ppb; milk – 50 ppb; cream, cheese – 5 ppb

**Recovery rate:** 85 ± 10%

**Application:** this kit can be applied for quantitative detection of DON in a range of samples including: grains, feed, milk, cream, cheese.

**Storage:** 2°-8°C for 12 months. Do not freeze.

**NOTE:** kit is for research purpose only.

**Kit components**

<table>
<thead>
<tr>
<th>Item</th>
<th>Specifications (96 wells)</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro ELISA Plate</td>
<td>96 (12 strips/8 removable wells)</td>
<td>4°C</td>
</tr>
<tr>
<td>Standard (calibrator)</td>
<td>6 vials/1 ml each</td>
<td>4°C</td>
</tr>
<tr>
<td>Sample/dilution buffer, 2x</td>
<td>50 ml (blue cap)</td>
<td>4°C</td>
</tr>
<tr>
<td>Wash solution, 20x</td>
<td>40 ml (blue cap)</td>
<td>4°C</td>
</tr>
<tr>
<td>DON Antibody solution</td>
<td>7 ml (blue cap)</td>
<td>4°C</td>
</tr>
<tr>
<td>HRP-DON enzyme conjugate</td>
<td>7 ml (red cap)</td>
<td>4°C</td>
</tr>
<tr>
<td>Substrate A</td>
<td>7 ml (brown cap)</td>
<td>4°C</td>
</tr>
<tr>
<td>Substrate B</td>
<td>7 ml (black cap)</td>
<td>4°C</td>
</tr>
<tr>
<td>Stop solution</td>
<td>7 ml (yellow cap)</td>
<td>4°C</td>
</tr>
</tbody>
</table>

**Important precautions before use**

1. Initial experiment using standards and a small number of samples is recommended.
2. We recommend duplicate well assay for standard and sample testing.
3. The room temperature below 20 °C as well as reagents not equilibrated to 20-25 °C will lead to a lower OD value. All solutions need to be well mixed.
4. Keep the microtiter plate dry before use.
5. Spin tubes so that all components are brought to the bottom of tubes.
6. Washing steps are of crucial importance to avoid false positive signals.
7. The microtiter plate should not be dry during the assay. A dry plate can inactivate active components in the wells and lead to non-linear standard curve.
8. Re-seal the unused microplate. HRP-labeled conjugate and TMB-substrate are photosensitive and are packaged in a protective opaque bottle. Store in the dark and return to storage after use. Avoid exposure to light.

9. The stop solution contains sulfuric acid solution, therefore avoid direct skin contact.

10. In case any solution will show signs of change in color, it cannot be used. Solution of a standard at 0 with OD value of less than 0.5 should not be used in an assay.

11. In case final reaction gives weak coloration, prolong incubation time, however, do not exceed 30 minutes.

12. Do not re-use tips and tubes to avoid cross contamination and do not use this kit once expiry date has passed.

13. Do not dilute, re-use or replace reagents or use different lot numbers combined together.

Caution when using TMB Substrate Solution:

1. Do not use a glass pipette to measure the TMB Substrate solution.

2. Do not cover the plate with aluminum foil or metalized mylar.

3. Do not return leftover of TMB Substrate back into the bottle.

4. Do not contaminate the unused TMB Substrate.

5. In case TMB Substrate Solution is blue, it cannot be used.

The following components are required but not supplied:

1. Microplate reader (450 nm or 630 nm).

2. Single and multi-channel pipettes and tips.

3. Distilled or de-ionized water, CH₃OH (100 %), CHCl₃, N-hexane.

4. Graphing software for analyzing the data.

5. Automated plate washer.

6. Absorbing paper.

7. Deionized or distilled water.

8. Timer. Microplate reader, homogenizer, nitrogen-drying device, vortex, centrifuge, balance (0.01 g).

Washing Method:
Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350 µl wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure two more times for a total of three washes.

Automated Washing:
Aspirate all wells, then wash plate 3x with 350 µl wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 minute. Avoid microbial contamination of equipment. Automated plate washers can easily become contaminated thereby causing assay variability.
Sample Extraction
Dilute CH3OH (30 ml CH3OH + 70 ml deionized water), mix properly

Sample dilution
10 ml of Sample diluent (2x) + 10 ml of distilled water, mix properly

Wash solution
Dilute 40 ml of washing solution (20x) with the deionized water to 800 ml, mix properly

Sample Preparation

Grains (corn, rice, wheat)
1. Weigh 2g of homogenized sample into 50 mL tube, add 10 ml Sample Extraction buffer and shake for 10 minutes.
2. Filter with Whatman No.paper, take up resulting supernatant.
3. Take up 50 µl of supernatant and add 950 µl of Sample Diluent, mix for 30 seconds.
4. Take 50 µl for analysis (sample dilution factor is 100).

Food Oil
1. Take 1g of oil into 50 ml tube, add 5 ml n-hexane, followed by 10 ml of sample extraction buffer and shake for 5 minutes.
2. Centrifuge at 4000 rpm/min for 10 minutes.
3. Take up 50 µl, add 450 µl of Sample Diluent and mix for 30 seconds.
4. Take 50 µl for analysis (sample dilution factor is 100).

Milk
1. 50 µl milk sample, add 450 µl of Sample Diluent and mix for 30 seconds.
2. Take 50 µl milk for analysis (sample dilution factor is 10).

Milk powder, cream, cheese
1. Weigh 5 g of homogenized sample into centrifuge tube, add 10 ml of CH3OH, shake for 5 minutes.
2. Centrifuge at above 4000 rpm/min for 10 minutes.
3. Take 2 ml of resulting supernatant into another tube, dry at 50°C with nitrogen.
4. Add 1m L C6H14, shake properly followed by addition of Sample Diluent, mix for 30 seconds.
5. Centrifuge at above 4000 rpm/min for 10 minutes.
6. Take up 50 µl lower layer for further analysis (sample dilution factor is 1).
ELISA Procedure

Note: Using standards and samples in duplicate will improve assay precision and accuracy.

1. Bring all reagents to the room temperature for at least 30 minutes. Mix well before use.
2. Take the required micro-well strips and plate frames. Re-seal the unused microplate, store at 2-8 °C until further use. Do not freeze.
3. Add 50 µl of sample or standard solution to separate duplicate wells, and add 50 µl Deoxynivalenol Antibody Solution into each well. Mix gently by shaking the plate annually, cover the plate with sealing tape to avoid evaporation and UV light, and incubate at 25°C for 20 minutes.
4. Pour liquid out of microwell, add 250 µl / well of diluted Wash solution for 15-30 seconds, repeat four to five times, then flap to dry (if there are the bubbles after flapping, cut them with tips).
5. Add 100 µl of the enzyme of DON HRP enzyme conjugate to each well, cover the plate with sealing tape, and incubate at 25°C for 10 minutes.
7. Take the same volume of substrate A and substrate B into a clean container, and mix them. Dispense 100 µl of the mixture into each well, cover the plate with sealing tape to avoid evaporation and UV light. Incubate for 10 minutes at 25°C.
8. Dispense 50 µl of stop solution and you will observe a color change in wells from blue to yellow.
9. Read and record the absorbance of the wells at 450 nm or 630 nm using a strip or plate reader within 30 minutes.

Result calculation

The percentage of absorbance values of standards (calibrators) and samples is the mean absorbance value of calibrators 2-6 or samples/ the mean absorbance value of calibrator 1, such as:

\[
\text{Percentage of absorbance value} = \frac{B}{B_0} \times 100\%
\]

B - the mean absorbance value of calibrators 2-6 or samples
B₀ - the mean absorbance value of calibrator 1

Quantitative interpretation requires graphing the concentration of the calibrators (X axis) versus the percentage of absorbance values of the calibrator (Y axis) on semi-log graph paper. A straight line is drawn through the calibrator points, and the sample absorbances are located on the line. The corresponding point on the Y axis is the concentration of the sample.
**Precautions**

Consistency of plate washing is influencing greatly reproducibility of the assay.

All incubations should be performed in controlled, constant temperatures. Performing an assay in temperature lower than 20°C will lead to lower standard OD value.

ELISA plate should never be allowed to turn dry, as this will result in non-linear standard curve and lack of reproducibility.

Samples and reagents must avoid exposure to light.

Each plate should be sealed by the cover membrane.

Kit cannot be used when expiry date is exceeded as well as reagents from other kits or batches should not be used together.

Unused ELISA plate must be stored correctly in a sealed bag.

In case any of reagents will change appearance, they cannot be used in an assay.

**Storage and expiry date**

The test kit is stable for 12 months at 2-8 °C in the dark. Do not freeze.