Tecna
R&D•Diagnostics - Biotechnology


# Enzyme immunoassay for the detection of Deoxynivalenol

## (code MD100/101)

***Celer* DON v2** is a kit prepared for an immunoenzymatic assay for the quantitative analysis of deoxynivalenol (DON).
The kit contains the materials and the procedure sufficient for 96 determinations ***(code MD100)*** or 48 determinations ***(code MD101)***, including standards.
A microtiter plate photometer or a strip photometer is required.

**Analysable samples**
Cereals and feed.

**Sample preparation**
- Cereals (maize, wheat) and feed: grinding, extraction in methanol-water, filtration, dilution (optional).
- Durum wheat: grinding, extraction in water, centrifugation, dilution.

**Assay time:** 20 minutes (sample preparation not included).

**Detection limit**
Maize, wheat and feed: 0.04 ppm
Durum wheat: 0.12 ppm

**Specificity**

| Compound | Cross-reactivity % |
|---|---|
| 3-acetyl-DON | >100 |
| DON | 100 |
| 15-acetyl-DON | 2 |

## 1 TEST PRINCIPLE

The assay is performed in plastic microplate, coated with specific anti-DON antibodies. In the premixing wells the enzyme labelled deoxynivalenol and the standard solutions or samples are mixed and then transferred into the anti-DON microtiter plate. During the first incubation, free deoxynivalenol in the standard solution /sample and enzyme-labelled deoxynivalenol compete for the anti-DON antibody binding sites on the solid phase. Any unbound enzyme conjugate and DON molecule are then removed in a washing step. The bound enzyme activity is determined adding a fixed amount of a chromogenic substrate. The enzyme converts the colourless chromogen into a blue product. The addition of the stop reagent leads to a colour change from blue to yellow. The absorbance is measured with a microplate reader at 450 nm. The colour development is inversely proportional to the deoxynivalenol concentration in the standard solution /sample.

## 2 PROVIDED REAGENTS

Microtiter plate: coated with anti-DON antibody, in an aluminium bag with a desiccant bag.
Code MD100: 96 wells (12 strips of 8 wells).
Code MD101: 48 wells (6 strips of 8 wells).
*As the strips are breakable, the wells can be used individually. For this purpose, it is sufficient to get out the wells from the sheath and to break the joint.*
Premixing microtiter plate: non-coated wells, blank.
Code MD100: 96 wells (12 strips of 8 wells).
Code MD101: 48 wells (6 strips of 8 wells).
MAIZE DON std: for the analysis of maize samples. 5 amber plastic vials containing 1.5 ml of: 0 ppm; 0.04ppm; 0.25 ppm; 1.25 ppm; 5 ppm of deoxynivalenol.
DON std: for the analysis of samples other than maize. 5 amber plastic vials containing 1.5 ml of: 0 ppm; 0.04ppm; 0.25 ppm; 1.25 ppm; 5 ppm of deoxynivalenol.
Enzyme conjugate: 1 amber plastic bottle.
Code MD100: 18 ml; code MD101: 12 ml.
Washing-buffer 10x: 1 plastic bottle containing 50 ml.
Developing solution: 1 amber plastic bottle containing 15 ml.
Stop solution: 1 plastic bottle containing 9 ml.

## 3 REQUIRED BUT NOT PROVIDED MATERIALS

***For sample preparation***
- Balance
- "Mycotoxin Extraction Solution A" code Tecna ME070 or methanol (wheat, maize, feed); methanol (Durum wheat)
- Distilled or deionized water
- NaCl (wheat, maize, feed)
- Mill (grinding)
- Shaker (optional)



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- Centrifuge like Labnet 512, cod. Tecna PC-084 (durum wheat) or filter paper (Whatman 1) (wheat, maize, feed)

***For assay implementation***

- 20-200 µl micropipettes with suitable tips
- 50-300 µl multichannel micropipette with suitable tips
- Absorbent paper
- Microtiter plate or strip reader equipped with a 450 nm filter.

## 4 WARNING AND PRECAUTIONS FOR THE USERS

- The product is for *in vitro* diagnostic use only.
- Some reagents contain preservative. The stop solution contains sulphuric acid and is corrosive. The standard solutions are toxic and inflammable because of methanol
- Handle the reagents with caution, avoiding contact with skin, eyes and mucous membranes.
- Safety data sheet are available on Tecna's web site.

## 5 HANDLING AND STORAGE INSTRUCTIONS

- Store the kit at +2/+8 °C and do not freeze components.
- Reseal the unused strips of the anti-DON microtiter plate in the bag together with the desiccant bag provided.
- Do not use components after the expiration date.
- Do not intermix components from different kit lots.
- Do not use photocopies of the instruction booklet. Keep always the instruction booklet that is included inside the kit.

## 6 SAMPLES PREPARATION

### 6.1 Wheat, maize and feed

- Mix carefully the sample to be analyzed in order to make it homogeneous.
- Finely grind the sample.
- Weigh 50 g of ground sample and add 10 g of NaCl. Add 250 ml of a solution of 70% methanol in distilled or deionized water. **Alternatively:** weigh 5 g of ground sample and add 1 g of NaCl. Add 25 ml of a solution of 70% methanol in distilled water.
- Shake thoroughly for 3 minutes. ATTENTION: it is suggested to shake manually or by a mild extraction system: it is possible to use magnetic stirrer, vortex or blender, but in same cases they can cause overestimation.
- Filter the sample and collect the filtrate. The sample is ready for a dosage range 0.04-5 ppm.
- If the samples is dosed >5 ppm, dilute the extract 5 times (1+4) in methanol 70%, in order to obtain a dosage range 0.2-25 ppm.

*It is suggested to weigh 50 gr in order to have a better representative analysis of the sample.*

### 6.2 Durum wheat

- Mix carefully the sample to be analyzed in order to make it homogeneous.
- Finely grind the sample.
- Weigh 50 g of ground sample and add 250 ml of. **Alternatively:** weigh 5 g of ground sample and add 25 ml of distilled water.
- Shake thoroughly for 15 minutes. ATTENTION: it is suggested to shake manually or by a mild extraction system: it is possible to use magnetic stirrer, vortex or blender, but in same cases they can cause overestimation.
- Filter the sample and collect the filtrate. ATTENTION: the filtration step could be very slow and it is suggested to let the sample settle down before filtration. As alternative, we suggest to centrifuge the sample at 3500g for 5 minutes and recover the supernatant.
- Dilute the supernatant 1:3 (1+2) with methanol 100% (es. 100ul of supernatant + 200 ul of methanol 100%). The sample is ready for a dosage range 0.12-15 ppm.
- If the samples is dosed >15 ppm, dilute the extract 5 times (1-4) in methanol 70%, in order to obtain a dosage range 0.6-75 ppm.

*It is suggested to weigh 50 gr in order to have a better representative analysis of the sample.*

## 7 WORKING SOLUTIONS PREPARATION

MAIZE DON std: ready to use.
DON std: ready to use.
Enzyme conjugate: ready to use.
Washing buffer: dilute the concentrate 1:10 (1+9) with distilled water; ATTENTION: in presence of crystals, bring the solution at room temperature and stir in order to solve them completely.
*The diluted washing buffer is stable at room temperature for 24 hours and at +2/+8°C for two weeks.*
Developing solution: ready to use; this solution is light sensitive: keep away from direct light.
Stop solution: ready to use. Attention: it contains 2 M sulphuric acid. Handle with care and in case of contact wash thoroughly with tap water.

## 8 ASSAY PROCEDURE

*8.1 Preliminary comments*

- Bring all reagents to room temperature before use, and keep them at room temperature at least for an hour.
- Return all reagents to +2/+8 °C immediately after use.
- Do not change the assay procedure, in particular:
  - do not prolong the first incubation time;
  - do not incubate the plate at a temperature higher than 25°C or lower than 18°C;
  - do not shake the plate during the incubations;
  - use for dispensing accurate and precise micropipettes with suitable tips.
- Once started, complete all the steps without interruption.
- The reproducibility of ELISA results depends largely upon the efficiency and uniformity of microwells washing; always keep to the described procedure.
- Use a single disposable tip for each standard and sample to avoid cross-contamination.
- Do not allow tips to contact the liquid already in the microwells.
- Avoid direct sunlight during all incubations. It is recommended to cover the microtiter plate without using sealing tapes.

### *8.2 Assay procedure*

1. Predispose the assay layout, taking into account that one well is required for each standard and sample; remove the wells not to be used from the anti-DON microtiter plate and replace them in



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the pouch with the desiccant gel and reseal the pouch very well using the clump provided.
Prepare an equal number of premixing wells.
ATTENTION: it is suggested to carry out no more than 48 determinations in each assay (standards included); if a multichannel pipette is not used, it is suggested to carry on no more than 16 determinations in each assay (standards included).

2. Add 100 µl of enzyme conjugate **in each premixing well**.
3. Add 50 µl of each standard/ sample into the corresponding premixing wells. ATTENTION: use only the standard solutions of the desired calibration curve, depending on the kind of sample. The standard/sample contain high percentage of methanol: take care to rinse the tip pipetting up and down the solutions before adding to the wells.
4. Using the micropipette, mix the content of each premixing well (pipette up and down three times) and immediately transfer 100 µl into the corresponding anti-DON antibody coated microwell.
ATTENTION: use new tips for each well to avoid cross-contamination.
5. Incubate 10 minutes at room temperature;
*Do not prolong the first incubation time and do not shake during incubation .*
6. Washing sequence
   - At the end of incubation, pour the liquid out from the wells.
   - Fill completely all the wells with washing buffer 1x using a squeeze bottle. Pour the liquid out from the wells. Repeat the washing sequence for a total of three times.
   - Remove the remaining droplets by tapping the microplate upside down vigorously against absorbent paper.

   *Do not allow the wells to dry out.*
7. Developing

– Add 100 µl of development solution to each well and mix thoroughly with rotatory motion for few seconds;

8. Incubate for 10 minutes at room temperature. Protect from light.
9. Add 50 µl of stop solution to each well micropipette and mix thoroughly with rotatory motion for few seconds.
10. Measure the absorbance at 450 nm. Read within 60 minutes.

*In case a strip reader is used, it is necessary to take out the strip from the frame and to remove the case round the wells.*

## 9 RESULTS CALCULATION

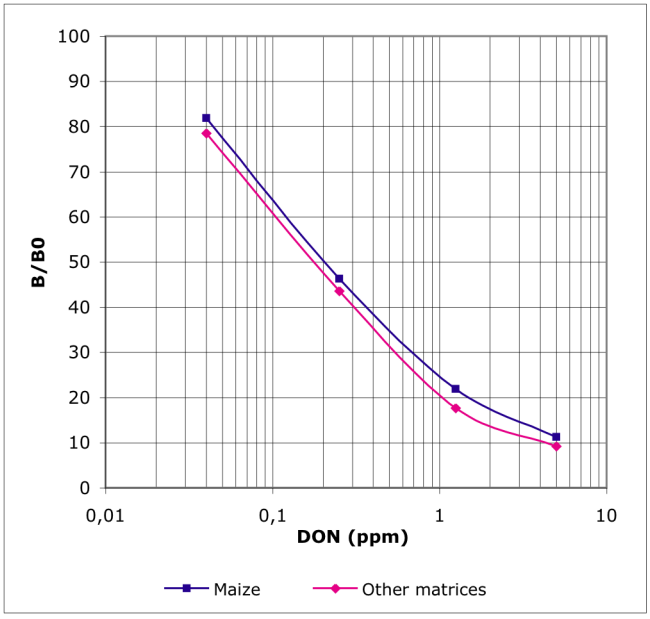
– Divide the absorbance value of each standard and sample by the absorbance of the Standard 0 ($B_0$) and multiply by 100; the Maximum Binding ($B_0$) is thus made equal to 100% and the absorbance values are quoted as percentage:

$$\frac{\text{standard (or sample) absorbance}}{\text{Standard 0 } (B_0) \text{ absorbance}} \times 100 = \frac{B}{B_0}$$

- Enter the $B/B_0$ values calculated for each standard in a semi-logarithmic system of coordinates against the DON standard concentration and draw the standard curve.
- Take the $B/B_0$ value for each sample and interpolate it to the corresponding concentration in the calibration curve, as follows:
  - wheat, maize and feed: the value read on the calibration curve corresponds to DON contamination in the sample, as standards concentration already considers the sample dilution factor;
  - durum wheat: multiply the value read on the calibration curve by a factor 3;
  - all the samples: if extract have been further diluted in order to obtain a larger dosage range, further multiply the results by a factor 5.

*For the calibration the "spline" curve fit or the "point to point" curve can be used. For calculation of the ELISA results using the "point to point" curve, Excel spreadsheets can be downloaded from the section "download" on Tecna's web site, www.tecnalab.com.*

## 10 EXAMPLE OF STANDARD CURVE



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## 11 RESULTS EVALUATION

After results elaboration, it is necessary to verify the assay performance. The verification is performed by comparison of obtained data with those given in kit specifications (paragraph 12). If the values are out from the specifications given, it is advised to control the expiry date of the kit, the wavelength of absorbance recording, as well as the procedure employed. If operation errors do not emerge, contact our technical assistance.
WARNING: substitution will be possible just in case of rendered kit. The kit must be conserved in its integral version and at the temperature indicated in this booklet.

## 12 KIT SPECIFICATIONS

### 12.1 Assay specification

| | |
|---|---|
| Bo absorbance | $\geq$ 0.7 $OD_{450nm}$ |
| $B/B_O$ 50% | 0.09-0.38 ppm |

### 12.2 Assay performance

| | |
|---|---|
| LOQ | Wheat, maize, feed: 0.125 ppm<br>Durum wheat: 0.25 ppm |

## 13 BIBILIOGRAPHY

Rapid and sensitive DON mycotoxin assay comparison on wheat, durum wheat and corn
F. Diana, G. Rosar, L. Persic and M. Paleologo. Food and Beverage Text Expo, 8-10 February 2011, Cologne, Germany.

## 14 LIABILITY

Samples evaluated as positive using the kit have to be re-tested with a confirmation method.
Tecna shall not be liable for any damages to the customer caused by the improper use of the kit and for any action undertaken as a consequence of results.
Tecna shall not be liable for the unsafe use of the kit out of the current European safety regulations.



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