Enzyme immunoassay for the detection of zearalenone
(code MZ470/MZ471)

Celer ZONv2 **ELISA kit** is a kit prepared for an immunoenzymatic assay for the quantitative analysis of zearalenone.
The kit contains the procedure and the materials sufficient for 96 determinations ***(code MZ470)*** or 48 determinations ***(code MZ471)*** including standards.
A microtiter plate photometer or a strip photometer is required.

Analysable samples
Cereals.

Sample preparation
Grinding, extraction in methanol-water, filtration or centrifugation, dilution (optional).

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

Detection limit
Cereals: 10 ppb.

Specificity	
Compound	**Cross-reactivity**
Zearalenone	100 %
α - zearalenol	42 %
β - zearalenol	12 %
α - zearalanol	71%
β–zearalanol	43%
Zearalanone	154 %

1 TEST PRINCIPLE

The assay is performed in plastic microwells that have been coated with anti-zearalenone antibodies. In the premixing wells the enzyme labelled zearalenone and the standard solutions or samples are mixed and then transferred into the anti-zearalenone microtiter plate. During the incubation, free zearalenone and enzyme-labelled zearalenone compete for the anti-zearalenone antibodies binding sites on the solid phase. Any unbound enzyme conjugate and zearalenone molecule is then removed in a washing step. The bound enzyme activity is determined by 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 zearalenone concentration in the sample.

2 PROVIDED REAGENTS

Premixing microtiter plate: non-coated wells, blank.
Code MZ470: 96 wells (12 strips of 8 wells)
Code MZ471: 48 wells (6 strips of 8 wells).
Microtiter plate: coated with anti-zearalenone antibody, in an aluminium bag with a desiccant bag.
Code MZ470: 96 wells (12 strips of 8 wells)
Code MZ471: 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.
Zearalenone standard: 5 amber plastic vials containing 1,5 ml of: 0 ppb; 10 ppb; 50 ppb; 200 ppb; 1000 ppb.
Enzyme conjugate: 1 amber plastic vial containing the solution. Cod.MZ470: 18ml, Cod.MZ471: 12 ml.
Washing-buffer 10x:1 plastic bottle containing 50 ml.
Developing solution: 1 amber plastic bottle containing 15 ml.
Stop solution: 1 glass vial containing 9 ml. White cap.

3 REQUIRED BUT NOT PROVIDED MATERIALS

- Methanol.
- NaCl
- Deionized or distilled water.

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Equipment
- 20-200 µl, 200-1000 µl precision micropipette with tips.
- 50-300 µl multichannel micropipette with tips
- Balance.
- Analytic mill (grinding).
- Optional: shaker or blender (like "Osterizer").
- Filter paper (Whatman 1) or Bench-centrifuge (Labnet 512, cod. Tecna PC-084).
- Microtiter plate or strip reader equipped with a 450 nm filter.

4 WARNING AND PRECAUTIONS FOR THE USERS

- 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 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 microtiter plate in the bag together with the desiccant bag provided.
- Do not use components after the expiration date.
- Do not intermix components between different kit lots.
- Do not use photocopies of the instruction booklet. Keep always to the instruction booklet that is included inside the kit.

6 SAMPLES PREPARATION

- 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 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.
- Blend or shake thoroughly for 3 minutes.
- Choose one of the followings: 1) filter the sample (Whatman 1) and collect the filtrate; 2) centrifuge the sample at 3500g for 5 minutes and transfer the supernatant. The filtrate/supernatant can be directly tested in the assay, for a dosage range of 10-1000 ppb. Otherwise, dilute the sample five times in 70% methanol to obtain a dosage range of 50-5000 ppb.

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

7 WORKING SOLUTIONS PREPARATION

Zearalenone standard: ready to use; mix before 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 2M sulphuric acid. Handle with care and in case of contact wash thorougly 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. 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-zearalenone microtiter plate and replace them in 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

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(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 correspondent premixing wells.
4. Using the micropipette, pipette up and down three times and immediately transfer 100 µl of the content from each premixing well into a corresponding anti-zearalenone 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 working washing buffer 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.
9. Add 50 µl of stop solution to each well and mix thoroughly with rotatory motion for few seconds.
10. Measure the absorbance at 450 nm against an air blank. 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

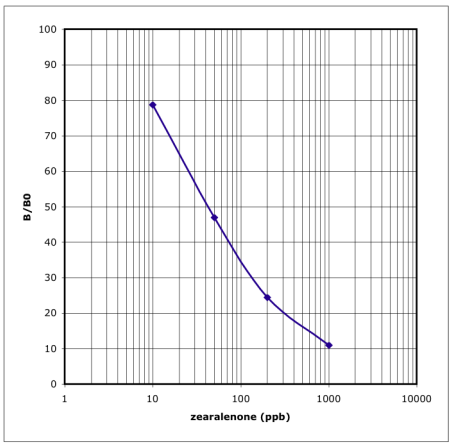
– Divide the absorbance value of each standard and sample by the absorbance of the Standard 0 (B_0) and multiply by 100; the Maximum Binding (B_0) is thus made equal to 100% and the absorbance values are quoted as percentage:

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

- Enter the B/B_0 values calculated for each standard in a semi-logarithmic system of coordinates against the zearalenone 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. Standards concentration (ppb) already considers the sample dilution factor. If the range of 50-5000ppb was used, multiply by five the values of concentrations obtained for the samples.

Please note: For calculation of the ELISA results, Excel spreadsheets can be downloaded from the section "download" in Tecna's web site, www.tecnalab.com.

10 EXAMPLE OF STANDARD CURVE



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. 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	≥ 0,7 OD_{450nm}
B/Bo 50%	14,6 - 73,5 ppb

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12.2 Assay performance

Cut-off	Maize, wheat: <10 ppb
LOQ	Maize, wheat: 20 ppb
Recovery % (spiking)	Maize, 100-500 ppb: 104±22% Wheat, 100-500 ppb: 105±6%

13 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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