Enzyme immunoassay for the detection of fumonisins

(code MF100/MF101)

Celer FUMO is an ELISA kit prepared for an immunoenzymatic assay for the quantitative analysis of fumonisins.
The kit contains the procedure and the materials sufficient for 96 determinations ***(code MF100)*** or 48 determinations ***(code MF101)*** including standards.
A microtiter plate photometer or a strip photometer is required.

Analysable samples
Cereals

Sample preparation
Grinding, extraction in methanol-water, filtration, dilution

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

Detection limit
0,75 ppm

Specificity

Compound	Cross-reactivity %
Fumonisin B_1	100
Fumonisin B_2	124 ± 11
Fumonisin B_3	100 ± 10

1 TEST PRINCIPLE

The assay is performed in plastic microwells that have been coated with anti-fumonisin antibody. In the premixing wells the enzyme-labelled fumonisin and the standard solutions or samples are mixed and then transferred into the anti-fumonisin microtiter plate. During the first incubation, free fumonisin in the standard solution /sample and enzyme-labelled fumonisin compete for the anti-fumonisin antibody binding sites on the solid phase. Any unbound enzyme conjugate and fumonisin molecule is 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 fumonisins concentration in the sample.

2 PROVIDED REAGENTS

Premixing microtiter plate: non-coated wells, blank.
Code MF100: 96 wells (12 strips of 8 wells)
Code MF101: 48 wells (6 strips of 8 wells).
Microtiter plate: coated with anti-fumonisin antibody, in an aluminium bag with a desiccant bag.
Code MF100: 96 wells (12 strips of 8 wells)
Code MF101: 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.
Fumonisin B_1 std: 5 amber glass vials containing 1,5 ml of standard equivalent to the following amounts of fumonisin in the sample: 0, 0.75, 4, 20, 60 ppm. White cap.
Enzyme conjugate: 1 amber glass vial, red cap.
Code MF100: 18 ml, code MF101: 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.

Equipment

- Balance.
- Grinder.
- Optional: shaker or blender (like “Osterizer”).
- Filter paper (Whatman 1).
- 20-200 µl micropipette with tips.
- 50-300 µl multichannel micropipette with tips (optional).
- Absorbent paper
- Microtiter plate or strip reader equipped with a 450 nm filter.

4 WARNING AND PRECAUTIONS FOR THE USERS

- For *in vitro* diagnostic use only.

Tecna S.r.l. - Area Science Park - Padriciano 99, Trieste, Italy
tel: +39 040 8325179, +39 040 3755341; fax +39 040 8326201, +39 040 3755343
e-mail: export@tecnalab.com web: http://www.tecnalab.com

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- Some reagents contain preservative. The stop solution contains sulphuric acid and is corrosive. The standard solutions are toxic because of methanol. The presence of fumonisin (carcinogenic compound) in the solutions does not make it more dangerous because the concentration is very low.
- 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 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.
- Filter the sample (Whatman 1) and collect the filtrate.
- Dilute the filtrate 1:20 with distilled water (50μl of extract + 950μl of water).

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

7 WORKING SOLUTIONS PREPARATION

Fumonisin B_1 std: 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 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-fumonisin 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 (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-fumonisin 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.

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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. 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

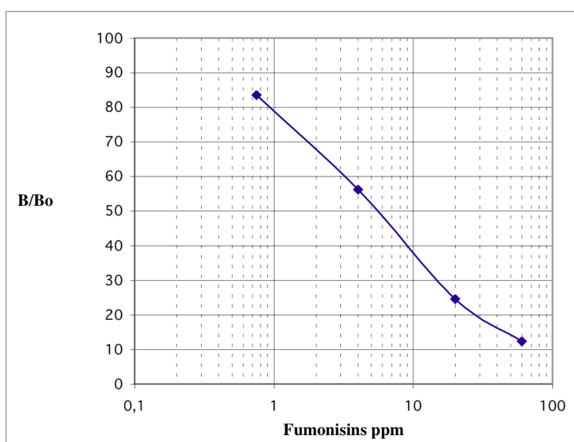
– Divide the absorbance value of each standard and sample by the absorbance of the Standard 0 (B_0) and multiply by 100; the Maximum Binding (B_0) is thus made equal to 100% and the absorbance values are quoted as percentage:

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

- Enter the B/B_0 values calculated for each standard in a semi-logarithmic system of coordinates against the fumonisin 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 (ppm) already considers the sample dilution factor.

For the calibration curve it is suggested to use the "point to point" curve or the "spline" curve fit or the linear regression, but not the "four parameters" logistic. For calculation of the ELISA results using the "point to point" curve, 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 specifications

Bo Absorbance	≥ 0,7 OD_{450nm}
B/Bo 50%	4 -8 ppm

12.2 Assay performance

LOQ	Maize: 1 ppm
Recovery % (range: 1,5-50 ppm)	– maize (spiked with fumonisin B_1): 97±10 – maize (naturally contaminated): 102±23

13 BIBLIOGRAPHY

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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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THE PRESENT DOCUMENT IS A FACSIMILE VERSION.
When performing the assay, please use the kit insert.

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