BZERO ZEA

Enzyme immunoassay for the detection of zearalenone (Cat.nr. HU0040027)

BZERO ZEA is a kit prepared for a competitive immunoenzymatic assay for the quantitative analysis of zearalenone.
The kit contains the procedure and the materials sufficient for 48 determinations.
For the result evaluation a microtiter plate or strip photometer is required (manual or automatic ELISA reader).

Type of samples that can be analyzed (matrices)
Cereals, feed, DDGS.

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

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

Detection limit
10 ppb

Specificity	
Compound	**Cross-reactivity (%)**
zearalenone	100
α - zearalenol	65±9
zearalanone	26±2
α - zearalanol	12±2
β - zearalenol	9±1
β - zearalanol	5±1

1. TEST PRINCIPLE

The assay is performed in plastic microwells that have been coated with anti- zearalenone antibodies. In the premixing wells the enzyme conjugate, the standard 0 or samples are mixed and then transferred into the anti-zearalenone microtiter plate.
During the first incubation, free zearalenone in the sample and enzyme-labelled zearalenone compete for the anti- zearalenone antibody binding sites on the solid phase. Any unbound enzyme conjugate and zearalenone 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 zearalenone concentration in the sample.

2. PROVIDED REAGENTS

Premixing microtiter plate: non-coated blank wells; 48 wells (6 strips of 8 wells).

Microtiter plate: plate coated with anti-zearalenone antibody, 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 frame and to break the joint.

Std. 0: 1 plastic vial containing 1.5 ml of 0 ppb of zearalenone.

ATTENTION: the standard zero is provided only. B/B_0 values of calibration curve (10 – 1000 ppb) are reported in the kit certificate of analysis.

Enzyme conjugate: 1 plastic vial containing 8ml.

Washing-buffer 10x: 1 plastic bottle containing 50 ml.

Developing solution: 1 plastic bottle containing 8 ml.

Stop solution: 1 glass vial containing 6 ml. White cap.

Component	Cat.nr. HU0040027 48 wells
Microtiter plate	48 wells (6 strip x 8 wells)
Std.0	1.5 ml
Enzyme conjugate	8 ml
Washing buffer 10x	50 ml
Developing solution	8 ml
Stop solution	6 ml

3. REQUIRED BUT NOT PROVIDED MATERIALS

- Distilled water
- Mycotoxin Extraction Solution A" cat.nr. Eurofins Tecna ME070 or, as an alternative, methanol
- NaCl

Equipment

- Balance
- For grinding: grinder or blender (like "Osterizer").
- For extraction (optional): shaker
- Filter paper (Whatman 1)
- 20-200 µl micropipette, tips
- 100-1000 µl micropipette, tips
- 50-300 µl multichannel micropipette, tips
- Microtiter reader, filter 450 nm

4. WARNING AND PRECAUTIONS FOR THE USERS

- The test for *in vitro* diagnostic use only.
- Some reagents contain solutions that may be identified as dangerous substance by the Regulation (EC) No 1272/2008. Please refer to *Material Safety Data Sheet* available on both the Eurofins Technologies (*eurofins-technologies.com*) and

Eurofins Technologies Hungary Kft., Fóti út 56., 1047 Budapest, Hungary
E-mail: technologies.hu@eurofins.com
Website: www.eurofins-technologies.com

Eurofins Tecna *(tecna.eurofins-technologies.com)* web sites.

Handle the reagents with caution, avoiding contact with skin, eyes and mucous membranes.

5. HANDLING AND STORAGE INSTRUCTIONS

- Store the kit at +2/+8 °C and never freeze.
- **Bring all reagents to room temperature before use (at least 1 hour).**
  ATTENTION: do not unseal the microplate until it reaches the room temperature.
- Reseal the unused strips of the microtiter plate in the bag together with the desiccant bag provided.
- Return all reagents to +2/+8 °C immediately after use.
- Do not use components after the expiration date.
- Do not intermix components from different kit lots.
- Do not use photocopies of the instruction booklet. Follow the original instruction booklet that is included with the kit.
- Do not change the assay procedure, in particular:
  - do not prolong the incubation times;
  - do not incubate the plate at temperatures higher than 25°C;
  - do not shake the plate during the incubations.
- Use accurate and precise micropipettes with suitable tips for dispensing.
- 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 solution and sample to avoid cross-contamination.
- Do not allow tips to contact the liquid already in the microwells.
- Avoid exposure to direct light during all incubations. It is recommended to cover the microtiter plate without using plate sealers.

6. SAMPLES PREPARATION

6.1 Cereals and feed

1) Mix carefully the sample to be analyzed in order to make it homogeneous.
2) Finely grind the sample.
3) Weigh the sample, choosing among the options described in the following table:

Sample	NaCl	Extraction solution
50 g	10 g	250 ml 70% methanol
5 g	1 g	25 ml 70% methanol
50 g	/	250 ml 70% methanol, 4% NaCl*
5 g	/	25 ml 70% methanol, 4% NaCl*

*** Preparation of extraction solution with 70% methanol and 4% NaCl:**
For 100 ml of solution: dissolve 4 gr of NaCl in 20 ml of deionized or distilled water, add 70 ml of methanol, then add deionized or distilled water to 100 ml.

4) Shake thoroughly for 3 minutes.
5) Filter the sample (Whatman 1) and collect the filtrate.
6) If the sample is dosed >1000 ppb, dilute the extract 5 times (1+4) in methanol 70%, in order to obtain a dosage range 50-5000 ppb.

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

6.2 DDGS

1) Mix carefully the sample to be analysed in order to make it homogeneous.
2) Finely grind the sample.
3) Weigh 50 g of ground sample and add 250 ml of a solution of 70% methanol in distilled water.

   Alternatively: weigh 5 g of ground sample and add 25 ml of a solution of 70% methanol in distilled water.
4) Shake thoroughly for 15 minutes.
5) Filter the sample (Whatman 1) and collect the filtrate.
6) If the sample is contaminated > 1000 ppb of zearalenone, dilute the extract five times (1+4) with 70% methanol, to obtain a 50-5000 ppb calibration range.

ATTENTION: For very highly contaminated samples it is suggested to repeat the analysis increasing the extraction time up to 60 minutes, in order to have a better accuracy of result.

7. WORKING SOLUTIONS PREPARATION

0 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 1 M sulphuric acid. Handle with care and in case of contact flush immediately with plenty of water.

8. ASSAY PROCEDURE

1) Predispose the assay layout, recording standard solutions and samples positions, taking into account that one well is required for each standard and sample. Prepare an equal number of premixing wells.
   ATTENTION: if a multichannel pipette is not used, it is advised to carry out no more than 16 determinations in each assay (standards included).
2) First incubation

- Add 100 µl of enzyme conjugate **in each premixing well**.

- Add 50 µl of standard 0 and each sample into the corresponding premixing wells. 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.
- 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-zearalenone antibody coated microwell.
- **ATTENTION**: use new tips for each well to avoid cross-contamination.
- Incubate 10 minutes at room temperature;
- Do not prolong the first incubation time and do not use automatic shakers.

3) Washing
   - 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 three (3) times.
   - Remove the remaining droplets by tapping the microplate upside down vigorously against absorbent paper.

Do not allow the wells to dry out.

4) Developing
   - Add 100 µl of developing solution to each well.
   - Mix thoroughly with rotatory motion for few seconds.
   - Incubate for 10 minutes at room temperature. Protect from direct light.
5) Add 50 µl of stop solution to each well and mix thoroughly with rotatory motion for few seconds.
6) Measure the absorbance at 450 nm.
7) Read within 15 minutes.

9. CALCULATION OF RESULTS

- Divide the absorbance value of each sample by the absorbance of the standard 0 (B_0) and multiply by 100; the standard 0 (B_0) is thus made equal to 100% and all the other absorbance values are expressed as percentage:

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

- Enter the B/B_0 values provided for each standard (10; 50; 400; 1000 ppb) in the kit certificate of analysis in a semi-logarithmic system of coordinates against the zearalenone standard concentration and draw the standard curve.
- Interpolate the B/B_0 value of each sample to the corresponding concentration from the calibration curve. Standards concentration (ppb) already considers the sample dilution factor.
- If the sample was 5 times further diluted in order to obtain a dosage range 50-5000 ppb, multiply the result read on the calibration curve by a factor 5.

Please note: to build the calibration curve use the "point to point" curve. Excel spreadsheet are available on website tecna.eurofins-technologies.com and can be downloaded directly from the bottom of the product page.

10. EVALUATION OF RESULTS

After processing the results, it is necessary to verify the assay performance. The verification is performed by comparison of obtained data with those given in kit specifications (see chapter 11).
If the values are outside the specifications given, then the results of the test are not assured, therefore the zearalenone concentration levels in the samples may not be valid.
In these cases it is advised to check the expiry date of the kit, the wavelength at which the reading was performed, as well as the procedure followed.
If operation errors are not identified as cause, contact our technical assistance.

WARNING: kit replacement will only be possible in case of return. The kit must be stored in its integral version at +2/+8°C.

11. KIT SPECIFICATIONS

11.1 Assay specification

B_0 absorbance	$\geq$ 0.7 OD_{450nm}

11.2 Assay performance

Matrix	Cut off (ppb)	LOQ (ppb)
Maize	<10	20
Wheat	18	25
Swine feed	40	50
DDGS	90	ND

12. LIABILITY

Samples evaluated as positive using the kit have to be re-tested with a confirmation method.
Eurofins Technologies Hungary 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.
Eurofins Technologies Hungary shall not be liable for the unsafe use of the kit out of the current European safety regulations.

13. LITERATURE

Rosar G., Diana F.: Master curve calibrated assays for cost-effective, reliable and consistent analysis of mycotoxins: a "B ZERO" review. Poster presentation at WMF meets ASIA, the12th Conference of The World Mycotoxin Forum, 2020, January 13-15, Bangkok, Thailandia.

Eurofins Technologies Hungary Kft., Fóti út 56., 1047 Budapest, Hungary
E-mail: technologies.hu@eurofins.com
Website: www.eurofins-technologies.com