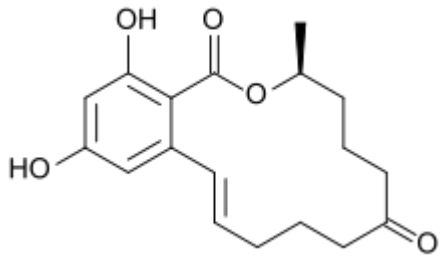
SENSI*Spec* ELISA Zearalenone 96 Tests

Enzyme Immunoassay for the Quantitative Determination of Zearalenone in Cereals, Milk, Nuts, Fruit and Beer/Gyle (Cat.nr. HU0030085)

Sensitivity (Pistachio)	0.04 ng/mL
Recovery	86 - 110%
Incubation Time	45 min

1. GENERAL INFORMATION



Zearalenone in addition to fumonisin, deoxynivalenol and other trichothecenes belongs to the fusarium toxins. These toxins are already produced on the field in consequence of a contact of the cereals by fusarium species. These moulds infect grain and other types of food like peanuts and beans already during their growth. When a considerable amount of zearalenone contaminated feed is taken up by cows, it can also be detected in their milk. Even in beer it could be found. Zearalenone shows a strong estrogen-like activity. Thus zearalenone can cause an enlargement of the uterus, diminution of the ovarian glands and even infertility. Zearalenone is one of the main contaminants of farm products, which can be taken up by humans and animals.

In the European Union the limits are 20 – 400 ppb for food products. Thus a monitoring of food and feed with respect to the concentration of zearalenone is obligatory.

The **SENSI*Spec* Zearalenone ELISA** represents a very highly sensitive detection system and is particulary capable of the quantification of zearalenone contaminations in cereals, milk, nuts, fruit and beer/gyle.

2. PRINCIPLE OF THE TEST

The **SENSI*Spec* Zearalenone** quantitative test is based on the principle of the enzyme-linked immunosorbent assay. An antibody directed against zearalenone is coated on the surface of a microtiter plate. Zearalenone containing samples or standards and a zearalenone-peroxidase conjugate are given into the wells of the microtiter plate. The conjugate competes with the zearalenone of the samples/standards for the limited number of antibody sites. After 30 minutes incubation at room temperature the wells are washed with diluted washing solution to remove unbound material. A substrate solution is added and incubated for 15 minutes, resulting in the development of a blue colour. The colour development is inhibited by the addition of a stop solution, and the colour turns yellow. The yellow colour is measured photometrically at 450 nm. The concentration of zearalenone is inversely proportional to the colour intensity of the test sample.

3. PRECAUTIONS

Full compliance of the following good laboratory practices (GLP) will determine the reliability of the results:

1) Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).

2) All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.

3) Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.

4) Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.

5) Use a separate disposable tip for each specimen to prevent cross-contamination.

6) All specimens and standards should be run at the same time, so that all conditions of testing are the same.

7) Do not mix components from different batches.

8) Do not use reagents after expiration date.

9) Check both precision and accuracy of the laboratory equipment used during the procedure (micropipets, ELISA reader etc.).

4. HEALTH AND SAFETY INSTRUCTIONS

1) Do not smoke or eat or drink or pipet by mouth in the laboratory.

2) Avoid contact of substrate and stop solution with skin and mucosa (possible irritation, burn or toxic-

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ity hazard). In case of contact, rinse the affected zone with plenty of water.

3) Handling and disposal of chemical products must be done according to good laboratory practices (GLP).

5. REAGENTS

The kit contains reagents for 96 determinations. They have to be stored at 2-8°C. Expiry data are found on the labels of the bottles and the outer package.

1) Microtiter plate consisting of 12 strips with 8 breakable wells each, coated with anti-zearalenone antibody.

2) Zearalenone Standards (0; 0.05; 0.15; 0.5; 1.5; 3.5 ng/mL): 6 vials with 1 mL each, dyed red, ready-to-use.

3) Conjugate (Zearalenone-Peroxidase): 6 mL, dyed red, ready-to-use.

4) Substrate Solution (TMB): 15 mL, ready-to-use.

5) Stop Solution (0.5 M H_2SO_4): 15 mL, ready-to-use.

6) Sample Diluent (PBS): 2 x 60 mL, dyed red, ready-to-use.

7) Washing Solution (PBS + Tween 20): 60 mL as 10x concentrate. Dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.

8) Plastic bag to store unused microtiter strips.

9) Instruction Manual.

6. ADDITIONAL INSTRUMENTATION AND REAGENTS (not provided)

Instrumentation

- 50, 100 and 500 µL- micropipets
- ELISA reader (450 nm)
- Centrifuge
- Ultra-Turrax, mixer, vortex

Reagents

- Double distilled water
- Methanol
- Hexan (Spices only)

7. SAMPLE PREPARATION

Cereals / Nuts / Fruit

- Grind sample to pass through a 20 mesh sieve and thoughly mix prior to sub-sampling.
- Suspend 20 g of sample in 100 mL of 70% methanol.
- Mix suspension for 5 minutes.
- Filter through Whatman #1 filter or alternatively centrifuge at a minimum of 3000 g for 5 minutes.
- Dilute 500 µL of filtrate/supernatant with 500 µL of sample diluent and test the sample in the ELISA.

Dilution factor = 10

Beer / Gyle / Milk

- Dilute an adequate volume of sample diluent with 35% methanol.
- Defat milk if applicable. Therefore the milk has to be centrifuged for 15 min at 4°C and at least 2000 g. Afterwards the upper fat layer should be removed.
- Carbonized beer samples should be preliminarily degassed by moderate heating.
- Cloudy beers (such as beer brewed from wheat) / gyle should preliminarily be sterile-filtered.
- Dilute 100 µL of prepared liquid sample with 900 µL sample diluent/methanol dilution.

Dilution factor = 10

In case of too high concentrated samples, an adequate volume of sample diluent is diluted with 35% methanol. The sample extracts have to be further diluted with this dilution.

8. PROCEDURE

1) Prepare samples as described above.

2) Pipet 100 µL standards or prepared samples in duplicate into the appropriate wells of the microtiter plate.

3) Add 50 µL of zearalenone-peroxidase conjugate into each well.

4) Incubate for 30 minutes at room temperature.

5) Wash the plate three times as follows: Discard the contents of the wells (dump or aspirate). Pipet 300 µL of diluted washing solution into each well. After the third repetition empty the wells again and remove residual liquid by striking the plate against a paper towel. The wash procedure is critical. Insuf-

ficient washing will result in poor precision and falsely elevated absorbencies.

6) Pipet 100 µL of substrate solution into each well.

7) Allow the reaction to develop in the dark (e.g. cupboard or drawer; the chromogen is light-sensitive) for 15 minutes at room temperature.

8) Stop enzyme reaction by adding 100 µL of stop solution (0.5 M H_2SO_4) into each well. The blue colour will turn yellow upon addition.

9) After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The colour is stable for 30 minutes.

9. CALCULATION OF RESULTS

1) Calculate the average optical density (OD 450 nm) for each set of reference standards or samples.

2) Construct a standard curve by plotting the mean optical density obtained for each reference standard against its concentration in ng/mL on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis. Alternatively the evaluation can be carried out by software. In this case the 4-parameter method should be preferred.

3) The diluted samples must be further converted by the appropriate sample dilution factor for calculating the sample concentration in ppb. The factors for each sample matrix are listed in the sample preparation section.

Example:

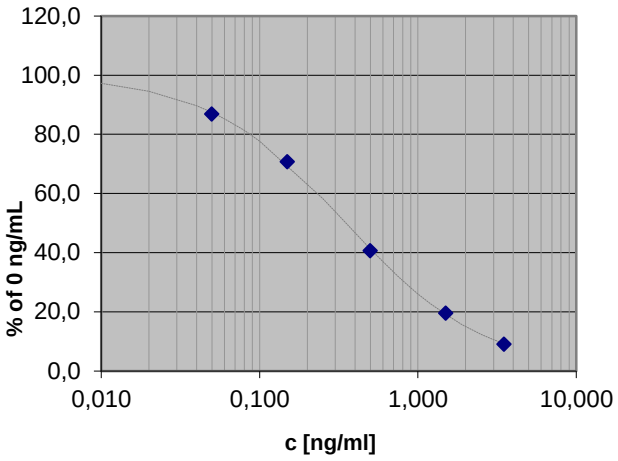
A milk sample prepared as described above results in 0.6 ng/mL. The concentration of the sample is calculated as follows:

C_{sample} = **0.6** (ng/mL) * **10** (ppb*ml/ng) = **6 ppb**

10. TYPICAL STANDARD VALUES

The following table contains an example for a typical standard curve. The binding is calculated as percent of the absorption of the 0 ng/mL standard. These values are only an example and should not be used instead of the standard curve which has to be measured in each new test.

Zearalenone (ng/mL)	(% binding of 0 ng/mL)
0	100
0.05	87
0.15	71
0.5	41
1.5	20
3.5	9



11. PERFORMANCE

11.1. Sensitivity

The limit of detection (LOD) of the **SENSI*Spec* Zearalenone test** is 0.043 ng/mL.

The limit of quantification (LOQ) of the **SENSI*Spec* Zearalenone test** is 0.130 ng/mL.

Validation experiments with common matrices resulted in the following LODs and LOQs [ppb].

Matrix	LOD	LOQ
Wheat	0.7	1.2
Barley	0.9	2.0
Rye	0.9	1.9
Rice	0.7	1.7
Corn	0.5	1.4
Banana	1.5	3.5
Peanut	3.9	7.6
Milk	2.8	5.8
Beer	0.4	1.0

11.2. Recovery

Wheat	86%
Barley	103%
Rye	105%
Oats	110%
Rice	93%
Corn	101%
Banana	86%
Peanut	90%
Milk	92%
Beer	94%

11.3 Linearity

The serial dilution of spiked samples (div. cereals, banana, peanut, milk and beer) resulted in a dilution linearity of 78-112%.

11.4 Precision

Intra-assay Precision	3-4%
Inter-assay Precision	3-13%

11.5 Cross-reactivity relative to Zearalenone (=100%)

α-Zearalanol	4%
β-Zearalanol	26%
α-Zearalenol	6%
β-Zearalenol	107%

12. REFERENCES

Suzuki T, et al. (2007) – Sensitive detection of estrogenic mycotoxin zearalenone by open sandwich immunoassay. Anal Sci, 23(1):65-69

Sang-Ho C, et al. (2012) – Production of highly group-specific monoclonal antibody against zearalenone and its application in an enzyme-linked immunosorbent assay. J Vet Sci, 13(2):119-125

Wang YP, et al. (2006) – Development of ELISA-kit for quantitative analysis of zearalenone. Wei Sheng Yan Jiu, 35(2):221-224

Rashedi M, et al. (2012) – Zearalenone contamination in barley, corn, silage and wheat bran. Toxic Ind Health, 28(9):779-782

Gao Y, et al. (2012) – Preparation of highly specific anti-zearalenone antibodies by using the cationic protein conjugate and development of an indirect competitive enzyme-linked immunosorbent assay. Analyst, 137(1):229-236

Burmistrova NA, et al. (2009) – Application o a new anti-zearalenone monoclonal antibodys in different immunoassay formats. Anal Bioanal Chem, 395(5):1301-1307

Thongrussamee T, et al. (2008) – Monoclonal based enzyme-linked immunosorbent assay for the detection of zearalenone in cereals. Food Add Contam Part, 25(8):997-1006

Bennett GA, et al. (1994) – Enzyme-linked immunosorbent assay for detection of zearalenone in corn, wheat and pig feed: collaborative study. J AOAC Int, 77(6):1500-1509

Vulic A, et al. (2012) – Analysis of naturally occurring zearalenone in feeding stuffs and urine of farm animals in Croatia. J Immunoassay Immunochem, 33(4):369-376

Hervás M, et al. (2009) – Electrochemical microfluidic chips coupled to magnetic bead-based ELISA to control allowable levels of zearalenone in baby foods using simplified calibration. Analyst, 134(12):2405-2411

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