Diagnostic Automation / Cortez Diagnostics, Inc.
IMMUNO DIAGNOSTICS

AccuDiag™ Zearalenone ELISA Kit

Cat# 5148-8

Test	Zearalenone ELISA
Recovery (spiked samples)	85-95 %
Total Time	~ 200 min.
Shelf Life	12-14 Months from the manufacturing date
Specificity	N/A
Sensitivity	0.25 ng/mL

INTENDED USE

Zearalenone belongs to the group of mycotoxins. Similar to other mycotoxins, zearalenone is a degradation product of the secondary metabolism. It is produced by different moulds of the Fusarium gender. 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 order to protect humans of illnesses caused by zearalenone, a qualitative and quantitative control of threatened food is essential, together with appropriate hygienic measures, which help to prevent the development of zearalenone.

The **Diagnostic Automation, Inc. Zearalenone ELISA** is a quick, economical and sensitive method to detect zearalenone in food. After an appropriate sample preparation, 40 samples can be tested in duplicate within 200 minutes.

TEST PRINCIPLE

The Diagnostic Automation, Inc. quantitative test is based on the principle of the enzyme linked immunosorbent assay. A zearalenone conjugate is bound on the surface of a microtiter plate. Zearalenone containing samples or standards and an antibody directed against zearalenone are given into the wells of the microtiter plate. Immobilized and free zearalenone compete for the antibody binding sites. After two hours incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A peroxidase conjugate directed against the zearalenone antibody is given into the wells and after hour incubation, the plate is washed again. Then a substrate solution is added and incubated for 20 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 indirectly proportional to the colour intensity of the test sample.

MATERIALS AND COMPONENTS

Materials provided with the test kits

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 zearalenone conjugate.
2. **Zearalenone Standards (0; 0.5; 2; 20; 200 ng/mL):** 5 vials with 1.0 mL each, ready-to-use.
3. **Anti-Zearalenone Antibody (rabbit):** 6 mL, dyed red, ready-to-use.
4. **Conjugate (anti-rabbit-IgG-HRP):** 15 mL, dyed red, ready-to-use.
5. **Substrate Solution (TMB):** 15 mL; ready-to-use.
6. **Stop Solution (0.5 M H_2S_{O4}):** 15 mL; ready-to-use.
7. **Sample Diluent (PBS):** 60 mL, ready-to-use.
8. **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 at 37°C for 15 minutes.
9. Two plastic foils to cover the strips during the incubation.
10. Plastic bag to store unused microtiter strips.
11. Instruction Manual.

Materials required but not provided

1. 50, 100 and 1000 µL-micropipettes
2. Microtiter plate shaker
3. ELISA reader (450 nm)
4. Mixer
5. Centrifuge
6. Horizontal shaker or magnetic stirrer

Reagents

1. Methanol
2. Distilled water
3. PBS (Phosphate buffered saline: 0.70 g/L $NaH_2PO_4x2H_2O$, 2.90 g/L $Na_2HPO_4x2H_2O$, 8.77 g/L NaCl, pH 7.2-7.4)

Sample Preparation

1. An appropriate amount of grain or nuts is crushed in a mixer for 3 minutes to produce a fine to medium-fine powder. If flour is used, the first step can be omitted.
2. 2 g of this powder are extracted with 10 mL methanol/PBS (60/40 v/v). This mixture is agitated for 30 minutes on a horizontal shaker (min. 120/minute). Alternatively also a magnetic stirrer can be used.
3. The total supernatant is centrifuged afterwards for 5 minutes at 3000 g.
4. The aqueous phase is diluted 1:10 in sample diluent, before being assayed in the Diagnostic Automation, Inc. **Zearalenone ELISA**.

ASSAY PROCEDURE

1. Prepare samples as described above.
2. Pipette 100 µL ready-to use standards or prepared samples in duplicate into the appropriate wells of the microtiter plate. Immediately add 50 µL zearalenone antibody into each well.
3. Cover the microtiter plate with a plastic foil and incubate for 120 minutes at room temperature on a microtiter plate shaker (or 180 minutes without shaker).
4. Wash the plate three times as follows: Discard the contents of the wells (dump or aspirate). Pipette 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. Insufficient washing will result in poor precision and falsely elevated absorbencies.
5. Pipette 100 µL of conjugate (anti-rabbit-IgG-HRP) into each well.

6. Cover the microtiter plate with a plastic foil and incubate for 60 minutes at room temperature on a microtiter plate shaker (or 90 minutes without shaker).
7. Wash the plate as outlined in 4.
8. Pipette 100 µL of substrate solution into each well.
9. Allow the reaction to develop in the dark (e.g. cupboard or drawer; the chromogen is light-sensitive) for 20 minutes at room temperature.
10. 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.
11. After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The colour is stable for 30 minutes.

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.
3. Using the mean optical density value for each sample, determine the corresponding concentration of zearalenone in ng/mL from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. The diluted samples must be further converted by the appropriate dilution factor (50 for the above described extraction). The factor is dependent on the sample preparation procedure employed.

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 every new test.

Zearalenone (ng/mL)	(% binding of 0 ng/mL)
0	100
0.5	90
2	81
20	42
200	15

PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity of the **Diagnostic Automation, Inc. Zearalenone ELISA** is 0.25 ng/mL.(based on the standard curve)

Recovery

The recovery of spiked grain samples was determined to 85-95%.

Intra-assay Precision

The intra-assay variation of the zearalenone test was determined to 3%.

PRECAUTION

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 (micropipette , ELISA reader etc.)

Health and safety instructions

1. Do not smoke or eat or drink or pipette by mouth in the laboratory.
2. Wear disposable gloves whenever handling patient specimens.
3. Avoid contact of substrate and stop solution with skin and mucosa (possible irritation, burn or toxicity hazard). In case of contact, rinse the affected zone with plenty of water.
4. Handling and disposal of chemical products must be done according to good laboratory practices (GLP).

REFERENCES

1. Teuscher, E. und Lindequist, U. (eds): *Biogene Gifte - Biologie, Chemie, Pharmakologie*. 2. Auflage, Gustav Fischer Verlag (1994).
2. Ueno, I. (ed): *Trichothecenes: chemical, biological and toxicological aspects*. Elsevier Science, ISBN 0-444-99661-3 (1983).
3. Scott, Peter, M. *Mycotoxins*. JAOAC, Vol. 70, No. 2, 276 - 281 (1987).

ISO 13485
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