Fungi Zearalenone (Rapid/HS) ELISA Kit

Enzyme Immunoassay for the rapid quantitative determination of Zearalenone in cereals and beer/gyle

Catalog number: ARG80820

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

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 Zearalenone (Rapid) ELISA Kit (HS) represents a highly sensitive detection system and is particulary capable of the rapid quantification of zearalenone contaminations in cereals and beer.

PRINCIPLE OF THE ASSAY

This assay employs the competitive quantitative enzyme immunoassay technique. An antibody binding protein is coated on the surface of a microtiter plate. Zearalenone containing samples or standards, a HRP-Zearalenone conjugate and an antibody directed against zearalenone are given into the wells of the microtiter plate. The conjugate competes with the zearalenone of samples / standards for the limited number of antibody sites. Simultaneously

the anti-zearalenone antibody is bound to the antibody-binding protein coated on the microtiter plate. After 10 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 10 minutes, resulting in the development of a blue color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The yellow color is measured at 450 nm. The concentration of zearalenone is indirectly proportional to the color intensity of the test sample.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody binding protein-coated microplate	12 strips x 8-well	4°C
Anti-zearalenone antibody	6 ml (ready to use)	4°C
HRP-zearalenone Conjugate	6 ml (ready to use)	4°C
Standards (0,10, 25, 75, 200, 500 ppb)	6 X 1 ml (ready to use)	4°C
Sample dilution buffer	2 X 60 ml (ready to use)	4°C
10x Wash Buffer	60 ml	4°C
TMB substrate	15 ml	4°C (Protect from light)
STOP solution	15 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the HRP-zearalenone conjugate before use.
- If crystals are observed in the 10X Wash buffer and Sample diluent buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

Cereals

- 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 double 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.

Beer / Gyle

- Dilute an adequate volume of sample diluent with 35 % methanol.
- 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 beer / gyle with 900 µl sample diluents/methanol dilution.

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.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X wash buffer into distilled water to yield 1X wash buffer.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Pipet 100 µl of standards or prepared samples in duplicate into the appropriate wells of the microtiter plate.
3. Add 50 µl of HRP-zearalenone into each well.
4. Add 50 µl of the anti-zearalenone antibody into each well.
5. Cover the microtiter plate with a plastic foil and incubate for 10 minutes at RT.
6. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1X wash buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
7. Add 100 µl of TMB mixture to each well. Incubate for 10 minutes at room temperature in dark.
8. Add 100 µl of Stop Solution to each well.
9. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal

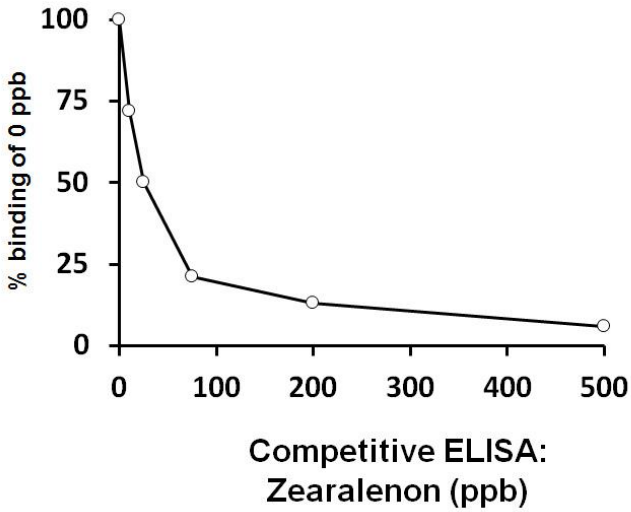
(X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The limit of detection (LOD) of the Zearalenone RAPID test is 5 ppb.

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

Wheat	7
Rye	8
Barley	8
Oats	8
Corn	8
Rice	7
Beer	7

The limit of quantification (LOQ) of the Zearalenone RAPID test is 10 ppb.

Due to the variety of sample matrices and their influence on the blank, results less than the LOQ should be treated as negative.

Specificity

Cross-reactivity	Relative to Zearalenone (=100 %)
α-Zearalanol	35%
β-Zearalanol	17%
α-Zearalenol	73%
β-Zearalenol	23%

Intra-assay and Inter assay precision

The CV value of intra-assay precision was 4-7% and the CV value of inter-assay precision was 5-13%.

Recovery

Wheat flour	90%
Oats flour	101%
Rice flour	99%
Corn flour	99%
Beer	100%