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Product no AS20 4393

ZEA | Zearalenone ELISA quantitation kit

Background

This ELISA assay utilizes the principle of competitive binding to measure the concentration of ZEA in analyzed samples. The ZEA (Zearalenone) has been pre-coated on the surface of the reaction wells. Samples containing an unknown amount of hormone, or standards, are mixed in the reaction well with a known amount of antibody to ZEA. During incubation, a competitive inhibition reaction occurs between the pre-coated ZEA and the hormone in the samples with the antibody specific to ZEA. Unbound hormone and plant extract are washed out of the reaction wells. Addition of HRP-conjugated goat anti-rabbit IgG antibody will visualize binding between anti-ZEA antibodies and ZEA. Substrate solution is added to the wells and the color develops in opposite to the amount of ZEA in the sample or standards. Reaction is stopped and the intensity of the color is measured at 450 nm.

Zearalenone is a RAL and F-2 mycotoxin produced by some *Fusarium* and *Gibberella* species. It is a potent estrogenic metabolite and is the primary toxin causing infertility, abortion, or other breeding problems, especially in swine. Zearalenone is found in a many cereal crops, such as maize, barley, oats, wheat, rice, sorghum as well as in bread all over the world.

The ZEA ELISA kit reagents

Assay plate	1 (96 wells)
Standard	5 x 1 ml
Antibody	1 x 7 ml
HRP-conjugate	1 x 7 ml
TMB Substrate	1 x 12 ml
Stop Solution	1 x 10 ml
Sample Diluent	3 x 50 ml
Wash Buffer (10x concentrate)	1 x 30 ml

Assay development time: 1-5 hours
Sensitivity: ≥ 0.15 µg/kg
Detection range: 0.15-4.05 µg/ml
Sample volume: 50 µl
Detection wavelength: 450 nm
Intra-assay precision (within assay): CV%<10%
Intra-assay precision (between assays): CV%<10%
Storage: 2-8°C (unopened kit) or 2-8°C for one month (opened kit)
Cross-reactivity: Zearalenone (ZEA) 100%
Sample type: feed, grain, *Glycine max* (fresh samples)

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Not provided in the kit but required to conduct the test

Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm **|** Incubator with stable incubation temperature up to 37°C **|** Squirt bottle, manifold dispenser or automated microplate washer **|** Absorbent paper for blotting the microtiter plate **|** 100 ml and 500 ml graduated cylinders **|** Deionized or distilled water **|** Pipettes and pipette tips **|** Test tubes for dilution series **|** Stirrer **|** Methanol **|** Ethanol

Important notes

The kit should not be used beyond the expiration date on the kit label. Reagents from different lots should not be mixed. In case analyzed samples are generating values which are higher than the highest standard, samples should be diluted, and the assay repeated. Variation in the assay can occur due to operator, pipetting technique, washing technique, incubation time and temperature as well as kit expiry date.

Samples: Samples should be added very carefully to avoid foaming and touching the well wall. For each step in the procedure, the total dispensing time for the addition of reagents or samples to the assay plate should not exceed 10 min. Cross contamination should be avoided by changing pipette tips between additions of each standard, sample and reagent.

Incubation: Proper adhesion of plate sealers during each incubation step has to be applied to ensure accurate results. Strips should not be let dry at any time. Incubation temperature and time must be kept constant with the optimal temperature being 25°C.

Washing: This step is critical, and the complete removal of liquid at each step is essential for a good performance of this assay. Any drops and fingerprints must be removed from a bottom of the plate. Insufficient washing will result in poor precision. In case of automated washing, addition of a 30 s soak period, following the addition of wash buffer and plate rotation by 180 degrees between each wash step, may improve assay precision.

Reaction development: Substrate should change from colorless or light blue to gradations of blue. In case the color is too intense Stop Solution should be added. Substrate solution can be easily contaminated. The liquid should remain colorless or light blue until added to the plate. Keep this solution away from any light.

Stop solution: The reaction is developed correctly if color development in the wells is from blue to yellow upon addition of the Stop Solution. Green color indicates that the Stop Solution was not thoroughly mixed with the substrate. In case of absorbance value of standard solution 0 being less than 0.5 this indicates its degeneration.

Safety measures

The Zearalenone ELISA quantitation kit Stop Solution is an acidic solution. Please use suitable protective gear for your eyes, hands, face, and clothes when handling this item.

Procedure instructions

Please read the whole manual carefully before proceeding with your experiment.

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Sample preparations

Feedstuff, feed, grain (Methanol)

Homogenize the material and weigh out 5 g (± 0.05 g) of sample. Add 25 ml of Methanol (70%) and vortex for 2 min. Centrifuge the mixture for 5 min at 4000 rpm and subsequently transfer 25 µl of supernatant into a new tube. Add 975 µl of Sample Diluent and shake well (sample dilution factor: 200). Use 50 µl of the sample for analysis.

Feedstuff, feed, grain (Ethanol)

Homogenize the material and weigh out 5 g (± 0.05 g) of sample. Add 25 ml of Ethanol (40%) and vortex for 2 min. Centrifuge the mixture for 5 min at 4000 rpm and subsequently transfer 25 µl of supernatant into a new tube. Add 975 µl of Sample Diluent and shake well (sample dilution factor: 200). Use 50 µl of the sample for analysis.

Samples prepared this way may be stored for up to one day at 2-8°C.

Manual

1. Bring all reagents and samples to room temperature before use (keep in RT for at least 30 min).

2. Centrifuge the samples. This assay is to be done on fresh samples or stored max 1 day at 2-8C. It is recommended that samples and standards are assayed in duplicate.

3. Prepare the samples and standards.

    a) Prepare the Washing Buffer (1x). In case crystals have formed, warm the concentrate to room temperature and mix gently until the crystals have dissolved. Prepare 100 ml of Wash Buffer (1x) by diluting 10 ml of Washing Buffer Concentrate (10x) into deionized or distilled water.

    b) Prepare the Standards. Centrifuge the standard vials at 6000-10000 rpm for 30 s. Standards are recommended to be used in duplicate.

Tube	S4	S3	S2	S1	S0
µg/kg	4.05	1.35	0.45	0.15	0

4. Determine the number of wells to be used. Unused wells should be put back into the Ziplock pouch and stored at 4°C.

5. Set one blank well without any solution. Add 50 µl of standard or sample to each well. Add samples gently to avoid foaming and be careful not to touch the well walls.

6. Add 50 µl of HRP-conjugate and 50 µl of Antibody (1x) to each well, except for the blank well. Mix thoroughly and incubate at 25°C for 15 min.

7. Aspirate the wells and wash the plate by filling the wells with Wash Buffer (1x, 250 µl in each well) using a squirt bottle, multi-channel pipette or auto-washer. Let it stand for 30 s, remove the liquid, and repeat the washing process three times for a total of four washes. After the fourth wash, remove all liquid and invert the plate against clean paper towels. Remove water and fingerprint on the bottom of the plate to avoid falsely reading results. The wash procedure is critical. Complete removal of liquid at each step is essential.

8. Add 100 µl of TMB Substrate to each well and mix well. Incubate at 25°C for 5 min in the dark.

9. Add 50 µl of Stop Solution to each well in the same order as the TMB substrate and mix thoroughly. The color will change from blue to yellow.

10. Read the optical density of each well within 5 min, at 450 nm.

Standard	µg/kg	Average OD
S4	4.05	0.194
S3	1.35	0.389
S2	0.45	0.911
S1	0.15	1.548
S0	0	1.926

Example of ZEA standard OD values. Note that developed color is inversely proportional to the amount of ZEA in the sample.

Results

There are two methods to judge the results: the first one (A) is the rough judgment, while the second (B) is the quantitative determination.

Note that the OD value of the sample has a negative correlation with Zearalenone (ZEN) in the sample.

A: Compare the sample average absorbance values with standards values, the Zearalenone (ZEN) concentration in the samples can be concluded. For example, the absorbance value of sample 1 is 0.799, the absorbance value of sample 2 is 1.129; absorbance values of standard are: 1.926, 1.548, 0.911, 0.389, 0.194 and the corresponding concentrations are: 0 µg/kg, 0.15 µg/kg, 0.45 µg/kg, 1.35 µg/kg, 4.05 µg/kg; then the Zearalenone (ZEN) in sample 1 and sample 2 are 0.45 µg/kg-1.35 µg/kg and 0.15 µg/kg-0.45 µg/kg. Lastly, the reader is multiplied by the corresponding dilution factor of each sample and the actual concentration of sample is obtained.

B: The software offered together will facilitate the calculation process, it is suitable for accurate and fast analysis of large-scale samples. Please contact us for more information. Note: Discard substrate with any color that indicates the degeneration of this solution. When the absorbance value of standard solution 0 is less than 0.5, this indicates its degeneration. The optimum reaction temperature is 25°C. A temperature that is too high or too low will result in changes in the absorbance value and detecting sensitivity.

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Related products

Aflatoxin B1 (clone 5C3) AS15 2851| Reactivity: Aflatoxin B1	Aflatoxin M1 (50 ug) AS14 2801| Reactivity: Aflatoxin M1	ZEA | Zearalenone (clone A3) AS15 2853 | Reactivity: Zearalenone