ZEN ELISA Kit

Catalog Number: orb411535

(USER MANUAL)

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique.

The coupling antigen is pre-coated on the micro-well stripes. The Zearalenone in the testing sample competes with the coupling antigens pre-coated on the micro-well stripes for the antibodies against Zearalenone. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the testing sample has a negative correlation with the content of Zearalenone in it. This value is compared to the standard curve and the content of the corresponding Zearalenone is subsequently obtained.

DETECTION RANGE

0.2 ppb-7.5 ppb.

SENSITIVITY

The minimum detectable dose of the kit is typically less than 0.2 ppb.

The sensitivity of this assay, or Lower Limit of Detection (LOD) was defined as the lowest concentration that could be differentiated from zero. It was determined the mean O.D value of 20 replicates of the zero standard added by their three standard deviations.

Limit of detection

Corn, wheat and grain corps	20 ppb
Feed and raw materials	40 ppb

Cross-reaction rate

Zearalenone	100%
Zearalanone	<13%
Zearalanol	<1%

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Recovery rate

Corn, wheat and grain corps	95±10%
Feed and raw materials	90±15%

PRECISION

Intra-assay Precision (Precision within an assay): CV%<10%

Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV%<10%

Three samples of known concentration were tested in twenty assays to assess.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

Reagent	Quantity
Assay plate	96 wells
Standard	5 x 1 ml
HRP-conjugate	1 x 6 ml
Antibody	1 x 6 ml
Substrate A	1 x 6 ml
Substrate B	1 x 6 ml
Stop Solution	1 x 6 ml
Sample Diluent (20 x concentrate)	1 x 40 ml
Wash Buffer (10 x concentrate)	1 x 40 ml
Adhesive Strip (For 96 wells)	4
Instruction manual	1

STANDARD CONCENTRATION

Standard	S0	S1	S2	S3	S4
Concentration (ppb)	0	0.2	0.6	2.5	7.5

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- An incubator which can provide stable incubation conditions up to 37°C±0.5°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.
- Methanol

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

Note:

- **Kindly use graduated containers to prepare the reagent.**
- Bring all reagents to room temperature (18-25°C) before use for 30min.
- Distilled water is recommended to be used to make the preparation for reagents or samples. Contaminated water or container for reagent preparation will influence the detection result.

REAGENT PREPARATION

1. **Wash Buffer(1x)-** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 40 ml of Wash Buffer Concentrate (10x) into deionized or distilled water to prepare 400 ml of Wash Buffer (1x).

2. **Sample Diluent(1x)-** Dilute 40 ml of Sample Diluent Concentrate (20x) into deionized or distilled water to prepare 800 ml of Sample Diluent (1x).

3. **50% Methanol**(immediately for use): Take equal volume of methanol and ddH2O, shake well.

4. **60% Methanol**(immediately for use): Dilute methanol with ddH2O(6:4):60 ml of methanol +40 ml ddH2O, shake well.

5. **90% Methanol**(immediately for use): Dilute methanol with ddH2O(9:1):90 ml of methanol +10 ml ddH2O, shake well.

Note:

1. Biorbyt is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.

2. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

3. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

SAMPLE COLLECTION AND STORAGE

A: Corn

(1) Weigh 5g of homogenized sample, add 50 ml of 50% Methanol and shake properly for 5 min.

(2) Centrifuge at 4000 r/min for 5 min.

(3) Transfer 0.5 ml supernatant and dilute with 4.5 ml of **Sample Diluent**(1x) at 10-fold.

(4) Take 50 µl for further analysis. Fold of dilution of the samples: 100

B: Wheat

(1) Weigh 5g of homogenized sample, add 50 ml of 50% Methanol and shake properly for 5 min.

(2) Centrifuge at 4000 r/min for 5 min.

(3) Transfer 0.5 ml supernatant and dilute with 4.5 ml ddH2O at 10-fold.

(4) Take 50 µl for further analysis. Fold of dilution of the samples: 100

C: Feed

(1) Weigh 3g of homogenized sample, add 30 ml of 60% Methanol and shake properly for 5 min.

(2) Centrifuge at 4000 r/min for 5 min.

(3) Transfer 0.2 ml supernatant into a new centrifugal tube and dilute with 3.8 ml ddH2O at 100-fold.

(4) Take 50 µl for further analysis. Fold of dilution of the samples: 200

D: Feed raw materials(corn gluten meal, DDGS, corn germ)

(1) Weigh 3g of homogenized sample, add 30 ml of 90% Methanol and shake properly for 5min.

(2) Centrifuge at 4000 r/min for 5 min.

(3) Transfer 0.2 ml supernatant into a new centrifugal tube and dilute with 3.8 ml ddH2O at 100-fold.

(4) Take 50 µl for further analysis. Fold of dilution of the samples: 200

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents and samples as directed in the previous sections.
2. Determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Add 50 µl of **Standard** or **Sample** per well. Standard and Samples need test in duplicate.
4. Add 50 µl of **HRP-conjugate** to each well. Then add 50 µl of **Antibody** to each well. Mix well and then incubate for 30 min at room temperature. Protect from light.

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5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with **Wash Buffer(1x)** (300 µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 30 seconds, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining liquid by aspirating ordecanting. Invert the plate and blot it against clean paper towels.

6. Add 50 µl of **Substrate A** and 50 µl of **Substrate B** to each well, mix well. Incubate for 15-20 minutes at room temperature. Keeping the plate away from drafts and other temperature fluctuations in the dark.

7. Add 50 µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.

8. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. (Recommend to read the OD value at the dual-wavelength: 450/630 nm within 5 min).

Note:

1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.

2. Samples or reagents addition: Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.

4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.

5. Controlling of reaction time: Observe the change of color after adding Substrates (e.g. observation once every 10 minutes). Substrates should change from colorless or light blue to gradations of blue. If the

color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.

6. Substrates are easily contaminated. Substrates should remain colorless or light blue until added to the plate. Please protect it from light.

7. Stop Solution should be added to the plate in the same order as the Substrates. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrates.

CALCULATION OF 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.

A:

Compare the sample average absorbance values with standards values, the Zearalenone concentration in the samples can be concluded. For example, the absorbance value of sample 1 is 0.659, the absorbance value of sample 2 is 1.525; absorbance values of standard are:2.101, 1.731, 1.284, 0.615, 0.229 and the corresponding concentrations are:0 ppb, 0.2 ppb, 0.6 ppb, 2.5 ppb, 7.5 ppb; then the Zearalenone in sample 1 and sample 2 are 0.6 ppb-2.5 ppb and 0.2 ppb-0.6 ppb. Lastly the reader is multiplied by the corresponding dilution factor of each sample and the actual concentration of sample is obtained.

B:

(1) The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

$$\text{Absorbency value(\%)} = \frac{B}{B0} \times 100\%$$

B——absorbance standard (or sample)

B0 ——absorbance zero standard

(2) To draw a standard curve: Take the absorbance value of standards as y-axis, logarithmic of the concentration of the Zearalenone standards solution (ppb) as x-axis.

The Zearalenone concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding Dilution factor of each sample followed, and the actual concentration of sample is obtained.

Note:

- Discard the substrate with any color that indicates the degeneration of this solution; when the absorbance value of standard solution 0 of less than 0.5 indicates its degeneration.
- The optimum reaction temperature is 25?, and too high or too low will result in the changes in the absorbance value and detecting sensitivity.