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Product Information

Zearalenone ELISA Kit

Catalog Number **SE120016**
Storage Temperature 2 to 8°C

TECHNICAL BULLETIN

Product Description

The Sigma-Aldrich® Zearalenone ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase direct competitive enzyme immunoassay.

A zearalenone-specific antibody optimized to react with zearalenone is coated to a polystyrene microwell. Toxins are extracted from a ground sample with 70% methanol. If zearalenone is present it will bind to the coated antibody. Subsequently, zearalenone bound to horse-radish peroxidase (HRP) is added and binds to the antibody not already occupied by zearalenone present in the sample or standard. After this incubation period, the contents of the wells are decanted, washed and an HRP substrate is added which develops a blue color in the presence of enzyme. The intensity of the color is directly proportional to the amount of bound conjugate and inversely proportional to the amount of zearalenone in the standard or sample. Therefore, as the concentration of zearalenone in the sample or standard increases, the intensity of the blue color will decrease. An acidic stop solution is added which changes the chromogen color from blue to yellow. The microwells are measured optically by a microplate reader with an absorbance filter of 450 nm (OD450). The optical densities of the samples are compared to the OD's of the kit standards and a result is determined by interpolation from the standard curve.

Intended Use

The Zearalenone ELISA kit is a competitive enzyme-linked immunoassay intended for the quantitative detection of zearalenone in cereal crops, such as maize, barley, oats, wheat, rice, and sorghum and also in bread.

Components

1. Zearalenone Microplate - 951ZEA01N: 96 wells (12×8-well strips) in a microwell holder coated with a mouse anti-zearalenone monoclonal antibody
2. Zearalenone Wine Standard – 6 vials, 953S2ZEA01N: 1.5 mL/vial of Zearalenone at the following concentrations 0.0, 0.3, 0.6, 1.2, 3.0, 10.0 ng/mL in aqueous solution.
3. Zearalenone HRP Conjugate - 954MZEA01N: 12 mL zearalenone conjugated to HRP in buffer with preservative.
4. Assay Diluent - 937AD001: 2x 12 mL proprietary sample diluent.
5. TMB Substrate - 916T001: 12 ml of stabilized 3,3',5,5'-tetramethylbenzidine (TMB).
6. Stop Solution - 946P001: 12 ml Acidic Solution.
7. PBST Wash Buffer Powder - 915X001: 1 packet of PBST. Bring to 1 liter with distilled water and store refrigerated.
8. Dilution Wells (Red): 96 wells non-coated (12 x 8 well strips) in a microwell holder. The wells are color coded red.

Reagents and Equipment Required but Not Provided.

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 100-200 µL volumes.
3. Collection container: 125 mL capacity.
4. Graduated cylinder: 100 mL
5. Methanol : Reagent Grade
6. Absorbent paper towels.
7. Graph paper or computer and software for ELISA data analysis.

Precautions and Disclaimer

1. This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.
2. Bring all reagents to room temperature (19-27 ºC) before use.
3. Consider all materials, containers and devices that are exposed to sample or standards to be contaminated with toxin. Wear protective gloves and safety glasses when using this kit.
4. HRP-labelled conjugate and TMB Substrate are photosensitive and are packaged in a protective opaque bottle. Store in the dark and return to storage.

Storage/Stability
Store reagents at 2-8 °C, and do not use beyond expiration date(s). Never freeze kit components.

Extraction Procedure
Note: The sample must be collected according to established sampling techniques.

1. Prepare the Extraction Solution (70% Methanol) by adding 30 mL of distilled or deionized water to 70 mL of methanol (reagent grade) for each sample to be tested.
2. Grind a representative sample to the particle size of fine instant coffee (50% passes through a 20 mesh screen).
3. Weigh out a 20 g ground portion of the sample and add 100 mL of the Extraction Solvent (70% methanol).
   Note: The ratio of sample to extraction solvent is 1:5 (w/v).
4. Mix by shaking in a sealed container or in a blender for three minutes.
5. Allow the particulate matter to settle, then filter 5-10 mL of the extract through a Whatman #1 filter paper (or equivalent) and collect the filtrate to be tested.
6. Dilute the sample extract 1:10 in 70% methanol (e.g. 0.1 mL + 0.9 mL)
7. Diluted sample is now ready for testing.

Assay Procedure
Note: It is recommended that a multi-channel pipettor be utilized to perform the assay. If a single channel pipettor is used, it is recommended that no more than a total of 16 samples and standards (2 test strips) are run.

1. Bring all the reagents to room temperature before use.
2. Place one mixing well in a microwell holder for each Standard and Sample to be tested. Place an equal number of Antibody Coated Microtiter wells in another microwell holder.
3. Dispense 200 µL of the sample diluent into each mixing well.
4. Using a new pipette tip for each, add 100 µL of each standard and prepared sample to appropriate mixing well containing diluent. Mix by priming pipettor at least 3 times.
   Note: Operator must record the location of each Standard and Sample throughout test.
5. Using a new pipette tip for each, transfer 100 µL of contents from each mixing well to a corresponding Antibody Coated Microtiter Well. Incubate at room temperature for 10 minutes. The mixing wells contain enough solution to run each standard and/or sample in duplicate if so desired.
6. Place sufficient HRP-conjugate (120 µL per standard/sample) in a trough and with a multichannel pipette add 100 µL of conjugate to the wells already containing standard/sample. Do not empty or wash the wells before adding the conjugate. The force of the addition of the second 100 µL to the first 100 µL causes sufficient mixing.
7. Continue the incubation for a further 10 minutes.
8. Decant the contents from microwells into a discard basin. Wash the microwells by filling each with PBST wash buffer, then decanting the wash into a discard basin. Repeat wash for a total of 3 washes.
9. Tap the microwells (face down) on a layer of absorbent towels to remove residual water.
10. Add 100 µL of TMB substrate to each antibody coated well and incubate for 10 minutes. Cover to avoid direct light.
11. Stop the reaction by adding 100 µL stop solution. The blue color will change to yellow.
12. Read the optical density (OD) of each microwell with a micro plate reader at 450 nm using an air blank or a differential filter of 630 nm.

Results
Construct a dose-response curve using either the unmodified OD values or the OD values expressed as a percentage of the OD of the zero (0.0) standard against the aflatoxin content of the standard. Unknowns are measured by interpolation from the standard curve.

The information contained on the label of a standard vial refers to the contents of that vial. However, the sample has been diluted at a 5:1 ratio with 70% methanol followed by a dilution of 10:1 in 70% methanol, and so the level of zearalenone shown by the standard must be multiplied by 50 in order to indicate the ng of zearalenone per gram of commodity (ppb) as follows:

Standard ng/mL	Commodity (ppb)
0.0	0.0
0.3	15.0
0.6	30.0
1.2	60.0
3.0	150.0
10.0	500.0

A 10 fold dilution of sample extract results in a standard curve of 15 - 500 ppb as shown above. Diluting the sample extract 20 fold will give a standard curve of 30 - 1000 ppb. If a sample gives a result outside the range of the standard curve it should be re-diluted and run again, taking the extra dilution into account when calculating the result.

Sensitivity
The sensitivity of the assay, calculated by taking multiple measurements of the zero standard and calculating 3 standard deviations below the mean is <15 ppb.

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