Deoxynivalenol ELISA Kit

Prod. No.: DEIA056
Pkg. Size: 48T

GENERAL DESCRIPTION

The Deoxynivalenol ELISA Test Kit is a competitive enzyme immunoassay for the quantitative analysis of Deoxynivalenol in Wheat, corn, feed, Beer, and wort . Deoxynivalenol (DON), also known as vomitoxin, belongs to the trichothecene group of mycotoxins and is formed by fungi of the genus Fusarium. DON occurs predominantly in grains such as wheat, barley, oats, rye, and maize. Due to their high cytotoxic and immunosuppressive properties these toxins pose a risk to human and animal health.
The **unique features** of the kit are:
1) Rapid extraction method for various samples with high recovery >80%.
2) High sensitivity (0.5 ppm) and low detection limit(0.2 ppm) .
3) A quick ELISA assay (less than 2 hours regardless of number of samples).
4) High reproducibility.

PRINCIPLE OF THE TEST

The method is based on an indirect competitive ELISA assay. The drug of interest has been coated in the plate wells. During the analysis, sample is added along with the primary antibody specific for the target drug. If the target is present in the sample, it will compete for the antibody, thereby preventing the antibody from binding to the drug attached to the well. The secondary antibody, tagged with a peroxidase enzyme, targets the primary antibody that is complexed to the drug coated on the plate wells. The resulting color intensity, after addition of substrate, has an inverse relationship with the target concentration in the sample.

REAGENTS AND MATERIALS PROVIDED

Microplate: 48 well polystyrene microplate (6 strips of 8 wells) coated with Deoxynivalenol;
Deoxynivalenol Standards (5): 0, 0.5, 1, 2, 4 ppm, 5 vials;
Antibody Solution: 4ml, 1 vial;
HRP Conjugate Antibody: 6ml, 1 vial;
Wash Solution (20×) Concentrate: 25 ml, 1 vial;
TMB Solution : 6ml, 1 vial;
TMB Stop Solution: 3ml, 1 vial;
Microtiter plate sealers
Plastic Sealable Bag

MATERIALS REQUIRED BUT NOT SUPPLIED

Equipment:
1. Validated microplate reader.
2. Homogenizer
3. Electronic balance
4. Centrifuger
5. Shaker for microtiter plates (optional)
6. Organomation
7. Vortex genie
8. Validated adjustable micropipettes, single and multichannel.
9. Timer

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STORAGE

Unopened Kit: Store at 2 - 8°C. Do not use past kit expiration date.
Opened/Reconstituted Reagents: TMB Solution; TMB Stop Solution; Wash Buffer; Antibody Solution; HRP-conjugate antibody
The above mentioned reagents should be stored for up to 1 month at 2 - 8°C.
Microplate Wells: Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8°C.

PRECAUTIONS

1. The kit should be equilibrated to room temperature (20-25℃) before opening any vials and starting the assay. It is highly recommended that the solutions be used as soon as possible after rehydration.
2. Do not mix or substitute reagents with those from other lots or sources.
3. 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.
5. Crystals could appear in the 20X wash solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solutions.
6. Keep TMB Substrate protected from light.
7. The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

SPECIMEN TREATMENT

Homogenized, centrifuged, evaporated, diluted for assay

ASSAY PROCEDURE

1. Add 50µl of the standard solutions or samples (sample extracts) into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
2. Add 50µl of antibody solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Incubate the strips for 30 minutes at room temperature. Wash the strips four times using the 1X washing buffer solution. Use at least a volume of 250 µl of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
3. Add 100µl of antibody solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for about 30 seconds. Be careful not to spill contents.
4. Incubate the strips for 30 minutes at room temperature.
5. After incubation, remove the covering and vigorously shake the contents of these wells into a sink. Wash the strips four times using the 1X washing buffer solution. Use at least a volume of 250 µl of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
6. Add 100 µl of TMB Solution into each well. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for about 30 seconds. Incubate the strips for 5-10 minutes at room temperature. Protect the strips from direct sunlight.
7. Add 50 µl of stop solution to the wells in the same sequence as for the substrate solution.
8. Read the absorbance at 450 nm using a microplate ELISA photometer within 5 minutes after the addition of the stopping solution.

EVALUATION

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-Parameter (preferred) or Logit/Log. For manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the %B/B_0 for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the %B/B_0 for each standard on the vertical linear (y) axis versus the corresponding Deoxynivalenol concentration on the horizontal logarithmic (x) axis on graph paper. %B/B_0 for samples will then yield levels in ppb of a Deoxynivalenol by interpolation using the standard curve. Samples showing lower concentrations of Deoxynivalenol compared to Standard 1 (50 ng/mL) are considered as negative. Samples showing a higher concentration than Standard 4 (400 ng/mL) must be diluted further to obtain accurate results.

WORKING SCHEME

The microtiter plate consists of 6 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

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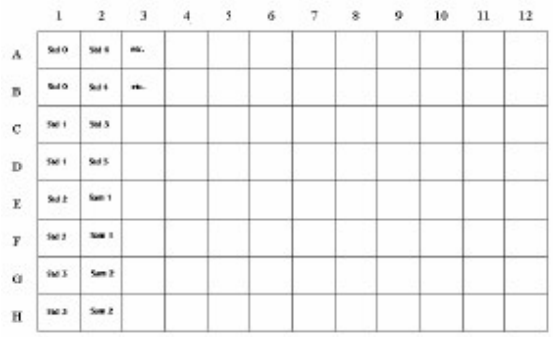
Std 0-Std 4: Standards
0, 0.5, 1, 2, 4 ppm
Sam1, Sam2, etc.: Samples



PERFORMANCE DATA

A standard curve can be constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ng/mL on a logarithmic curve.
Relative absorbance (%) = absorbance standard (or sample) x 100/ absorbance zero standard.
Use the mean relative absorbance values for each sample to determine the corresponding concentration of the tested drug in ng/mL from the standard curve.
The ELISA sensitivity is 0.5 ppm, and the range of the standard curve is 0.5-4 ppm.

INDICATIONS OF INSTABILITY OR DETERIORATION OF THE REAGENTS

1. Values of the Positive or Negative controls, which are out of the indicated Quality control range, are indicator of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the samples must be retested. In case of constant erroneous results classified as due to deterioration or instability of the reagents, immediately substitute the reagents with new ones, or contact our technical support for further assistance.

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