Aflatoxin ELISA Kit

Prod. No.: DEIA054
Pkg. Size: 96T

GENERAL DESCRIPTION

The Aflatoxin ELISA Test Kit is a competitive enzyme immunoassay for the quantitative analysis of Aflatoxin in feed, rice, maize, beans, peanuts, peanuts butter, cooking oil and other sample. Aflatoxin B1 is a toxin produced by fungus from the Aspergillus genus. The toxin is carcinogenic in humans and can also cause other health effect, particularly liver problems. The fungus are found frequently in nature and can readily contaminate crops before harvest or during storage. It is common in moist soils and decaying vegetation. There are more than 13 different subtypes of Aflatoxin with B1 being the most toxic. The condition is most common in poorly developed countries where there are insufficient controls on the presence of Aflatoxin in food.
The **unique features** of the kit are:
1) Rapid extraction method for various samples with high recovery 83~106%.
2) High sensitivity (0.05 ng/g or ppb) and low detection limit.
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 a competitive ELISA assay. The antibody of interest has been coated in the plate wells. During the analysis, sample is added along with the enzyme conjugates 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: 96 well polystyrene microplate (12 strips of 8 wells) coated with Aflatoxin antibody;
Aflatoxin B1 Standards (6): 0, 0.05, 0.15, 0.3, 0.9, 2.7ppb, 6 vials;
Aflatoxin Enzyme Conjugates: 7ml, 1 vial;
Wash Solution (10×) Concentrate: 30 ml, 1 vial;
TMB Solution A: 7ml, 1 vial;
TMB Solution B: 7ml, 1 vial;
TMB Stop Solution: 7ml, 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 A; TMB Solution B; TMB Stop Solution; Wash Buffer ; 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 10× 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 enzyme conjugate solution to the individual wells successively using a multi-channel pipette or a stepping pipette.
3. 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 three 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. Dispense 50 µl of TMB Solution A and 50 µl TMB Solution B 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 15 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 Aflatoxin B1 concentration on the horizontal logarithmic (x) axis on graph paper. %B/B_0 for samples will then yield levels in ppb of a Aflatoxin B1 by interpolation using the standard curve. Samples showing lower concentrations of Aflatoxin B1 compared to Standard 1 (0.05 ng/mL) are considered as negative. Samples showing a higher concentration than Standard 5 (2.7 ng/mL) must be diluted further to obtain accurate results.

WORKING SCHEME

The microtiter plate consists of 12 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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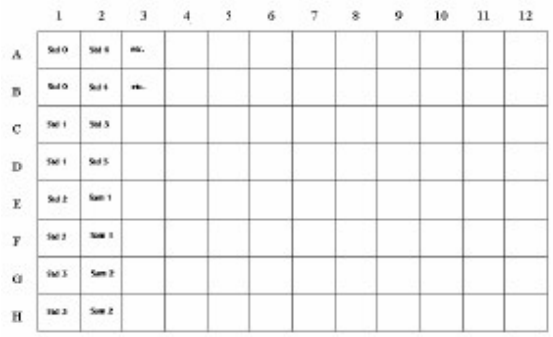
Std 0-Std 5: Standards
0, 0.05, 0.15, 0.3, 0.9, 2.7ppb
Sam1, Sam2, etc.: Samples



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 0.05 ppb, and the range of the standard curve is 0.05-2.7 ppb.

Specificity (Cross-Reactivity):

Aflatoxin B1.....100%
Aflatoxin B2.....105%
Aflatoxin G1.....96%
Aflatoxin G2.....28%

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.

2. If after mixing of the TMB Solution A and B into the wells, the color of the mixture turns blue within few minutes, do not continue carrying out the testing and replace the reagents with fresh ones.

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