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User's Manual

Sterigmatocystin ELISA Kit

REF **DEIANJ08**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Sterigmatocystin(ST) Kit is a competitive ELISA for the quantitative analysis of sterigmatocyst in grain and grain products.

General Description

Sterigmatocystin is a toxic metabolite structurally closely related to the aflatoxins, and consists of a xanthone nucleus attached to a bifuran structure. Sterigmatocystin is mainly produced by the fungi Aspergillus nidulans and A. versicolor. It has been reported in mouldy grain, green coffee beans and cheese although information on its occurrence in foods is limited.

Principles of Testing

This kit is based on direct-competitive ELISA technology. The microtiter wells are coated with ST coupling antigen. ST in the sample competes with the antigen coated on the microtiter plate for the enzyme conjugate antibody. After incubation, TMB substrate is used to show the color. Absorbance of the sample is negatively related to the ST concentration in samples, after comparing with the Standard Curve, multiplied by the dilution factor, ST quantity in the sample can be calculated.

Reagents And Materials Provided

Microtiter plate with 96 wells coated with antigen

Standard solutions(6 bottles)

Enzyme conjugate

Solution A

Solution B

Stop solution

Wash solution

Diluent solution

Manual

Materials Required But Not Supplied

1. Laboratory quality distilled or deionized water.
2. Graduated cylinder, 100 mL.
3. Glassware for sample extraction and extract collection.
4. Filter paper, Whatman GF/A or equivalent
5. Pipet with disposable tips capable of dispensing 50 µL.

6. Multi-channel pipet; 8 channel capable of dispensing 50 and 100 µL or Eppendorf Repeater pipette and tips for dispensing 50 and 100 µL.
7. Paper towels or equivalent absorbent material.
8. Microwell plate or strip reader with 450nm filter.
9. Timer
10. Blender

Storage

Store at 2 - 8°C.

Specimen Collection And Preparation

1. Grind samples to pass a 20 mesh sieve and thoroughly mix prior to sub-sampling. Samples not being immediately analyzed should be stored refrigerated.
2. Weigh 50 g ground sample and 5.0 g NaCl and transfer to clean blender jar.
3. Add 100 mL of 80% Methanol/water to the jar.
4. Blend for 1 minute in a high-speed blender.
5. Filter a minimum of 10 mL through a glass fiber filter.
6. Dilute 5 mL of extract with 20 mL of water and mix thoroughly.
7. Filter through a glass fiber filter.

Assay Procedure

Note:

Make sure all reagents and micro wells are all at roomtemperature (20-25°C).

Return all the rest reagents to 2-8°C immediately after used.

Washing the microwells correctly is an important step in the process of assay; it is the vital factor to the repetitiveness of the ELISA analysis.

Avoid the light and cover the microwells during incubation.

1. Take all reagents out at room temperature (20-25°C) for more than 30min, shake gently before use.
2. Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.
3. The diluted wash solution should be rewarmed to be at room temperature before use.
4. Number: Numbered every microwell positions and all standards and samples should be run in duplicate. Record the standards and samples positions.
5. Add standard /sample, enzyme conjugate antibody: Add 50µl of standard solution or prepared sample to corresponding wells. Add 50µl of enzyme conjugate. Mix gently by rocking the plate manually and incubate for 30min at 37°C with cover.
6. Wash: Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250µl diluted wash solution at interval of 10s for 4-5 times. Absorb the residual water with absorbent paper (the

rest air bubble can be eliminated with unused tip).

7. Coloration: Add 50µl of solution A and 50µl of solution B to each well. Mix gently by rocking the plate manually and incubate for 10min at 37°C with cover.
8. Measure: Add 50µl of the stop solution(kit provided) to each well. Mix gently by rocking the plate manually and measure the absorbance at 450nm (It's suggested measure with the dual-wavelength of 450/630nm. Read the result within 5min after addition of stop solution.)

Calculation

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.

Absorbance (%)=B/B0×100%

B ——absorbance standard (or sample)

B0 ——absorbance zero standard

To draw a standard curve: Take the absorbance value of standards as y-axis, semi logarithmic of the concentration of the ST standards solution (ppb) as x-axis. The ST concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution rate of each sample followed, and the actual concentration of sample is obtained.

Specificity

Sterigmatocystin 100%

Precautions

1. The mean values of the absorbance values obtained for the standards and the samples will be reduced if the reagents and samples have not been regulated to room temperature (20-25°C).
2. Do not allow microwells to dry between steps to avoid unsuccessful repetitiveness and operate the next step immediately after tap the microwells holder.
3. Shake each reagent gently before using.
4. Keep your skin away from the stop solution for it is 0.5M H2SO4 solution.
5. Don't use the kits out of date. Don't exchange the reagents of different batches, or else it will drop the sensitivity.
6. Keep the ELISA kits at 2-8°C, do not freeze. Seal rest microwell plates Avoid straight sunlight during all incubations. Covering the microtiter plates is recommended.