Version 20-11/24

User's Manual

Ochratoxin-A ELISA Kit

REF **DEIA4010**

Σ **96T**

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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.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The Ochratoxin-A ELISA Kit is intended for the quantitative detection of ochratoxin-A levels in grains, cereals, coffee, and other commodities including animal feeds.

General Description

Ochratoxin-A is a toxic metabolite produced by several molds of the Aspergillus flavus and Penicillium genera, including Aspergillus ochraceus. The fungal species has the potential to produce ochratoxin-A, a known nephrotoxin and carcinogen. It has been frequently detected in human foods and animal feed, mainly in cereal products, although a range of commodities has been reported to contain the toxin. In humans, exposure to ochratoxin-A has been linked with Balken endemic nephropathy (BEN), a chronic kidney disease associated with tumors of the renal system. In animals, impairment of renal function has been reported in swine. In turkeys and chickens symptoms included retarded growth, decreased feed conversion, nephropathy and mortality. Feed refusal has also been observed in turkeys. A decrease in egg production and shell quality was reported in both turkeys and chickens.

Principles of Testing

The enzyme immunoassay for ochratoxin-A is based on the competition between the ochratoxin-A in the sample and the Ochratoxin-A-Horseradish Peroxidase conjugate, for binding to antibody directed against ochratoxin-A, coated onto microwells. The sample containing the ochratoxin-A, and the Ochratoxin-A-Horseradish Peroxidase conjugate, when added to the microtiter wells, compete for binding to a limiting number of antibody sites. After incubation, each well is rinsed in order to remove non-bound components. The bound enzymatic activity is then measured by the addition of a chromogenic substrate. If no or small amount of ochratoxin-A is present in the sample more enzyme labeled ochratoxin-A will bind the antibody on the solid surface. On the other hand, if large or significant amount of ochratoxin-A is present in urine sample, less enzyme labeled ochratoxin-A will bind to the antibody, producing less color signal. Therefore, the intensity of the color developed is inversely proportional to the concentration of ochratoxin-A in the sample. The concentration is calculated on the basis of a standard curve.

Reagents And Materials Provided

Component	Amount
96-wells microtiter plate (#S). Twelve strips of 8 detachable wells, coated with Anti-Ochratoxin-A antibody.	96 (8x12) wells
Calibrators containing 0, 0.5, 2.0 and 8.0 ng/mL of Ochratoxin-A.	0.45 mL x 4
Ochratoxin-A-Horseradish Peroxidase Conjugate (ORT-HRP) (#3).	10.5 mL
Stabilized tetramethylbenzidine (TMB) substrate (#5). Ready to use.	10.5 mL
Wash Buffer (10x PBS-Tween) (#6). Dilute 10 fold with distilled or deionized water to 150 mL prior to use.	15 mL
Stop Solution, 3 N HCl (#7).	10.5 mL

Materials Required But Not Supplied

Pipetters capable of delivering 25 µL and 100 µL.

Microtiter plate reader (wavelength 450 nm).

Plate washer or squeezable wash bottle.

Timer.

Absorbent paper towels.

Storage

All reagents of the kit are stable, if stores at 2 - 8°C, until the expiration date stated on the kit.

Assay Procedure

Let the components of the kit equilibrate to room temperature before use.

1. Carefully add 25 µL of standard or samples to the bottom of each well. Slightly tap the side of the strip holder to evenly distribute the sample.
2. Avoid touching the well with pipette tip and add 100 µL of ORT-HRP conjugate (#3) to each well. Slightly tap the side of the strip holder to properly mix the sample and enzyme conjugate.
3. Incubate at room temperature for 30 minutes.
4. After incubation, dispose the solution in the wells by inverting and shaking. Wash microtiter wells 3 times with wash buffer to remove the non-bound conjugate. Washing may be done manually as follows: use squeeze bottle to fill wells gently with wash buffer, dumping the wells between each wash by inverting and shaking. After the third wash, tamp holder onto a piece of absorbent paper.
5. Add 100 µL of TMB substrate (#5) to each well and incubate at room temperature for 15 min. To avoid contamination, place the needed amount of substrate into a test tube and dispense only from the tube itself.
6. Add 100 µL of Stop Solution (#7) to each well and tap the strip holder for proper mixing.
7. Read absorbance at 450 nm using an ELISA reader.

Simplified Assay Procedure

1. Add sample or standard (25 µL).
2. Add enzyme conjugate (100 µL). 30 min at RT.
3. Wash 3×.
4. Add TMB (100 µL), wait for 15 min. at RT.
5. Add stop solution (100 µL) and read at 450 nm.

Calculation

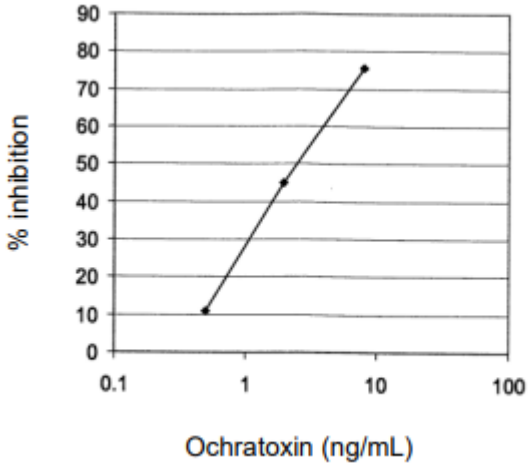
1. Calculation

   Average the absorbance (ODs) for each standard concentration of ochratoxin-A including 0 ng/mL (OD0).

   % of Inhibition = 100 - (ODs / OD0) ×100

2. Plot values of % of Inhibition, step 1 (b), against their corresponding concentrations on Log10 paper.
3. Calculate ochratoxin-A concentration in the sample by interpolation and multiply by the sample' s dilution factor to obtain the actual quantity of ochratoxin-A.

   Ochratoxin-A Inhibition curve



Precautions

Reagent are for in vitro research use only.

1. Store reagents at 2 to 8 °C, and do not use beyond expiration date. Never freeze kit components.
2. Do not return unused reagents back into their original bottles. The assay procedure details volumes required.
3. Samples tested should have a pH of 7.0 (± 1.0). Excessive alkaline or acidic conditions may affect the test results.
4. The stop solution contains acid. Do not allow to contact skin or eyes. If exposed, flush with water.
5. Consider all materials, containers and devices that are exposed to sample or standards to be contaminated with toxin. Wear protective gloves and safely glasses when using this kit.
6. Dispose of all materials, containers and devices in the appropriate receptacle after use.