Version 16-05/24

User's Manual

Ochratoxin A ELISA Kit

REF **DEIA6849A**

Σ **96T**

RUO

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

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

This datasheet establishes procedures for determining ochratoxin A in corn, grain, processed grain products and feed.

General Description

The mycotoxin ochratoxin A is formed by fungi of the species Aspergillus and Penicillium. Apart from a marked nephrotoxicity, ochratoxin A displays hepatotoxic, teratogenic, carcinogenic and immunosuppressive properties. There is a risk to human health not only through the intake of contaminated foods of vegetable origin, but also through foods of animal origin.

Principles of Testing

This handbook establishes procedures for determining ochratoxin A in corn, grain, processed grain products and feed. The test is a competitive direct ELISA that provides exact concentrations in parts per billion (ppb). Free toxin in the sample and controls competes with enzyme-labeled toxin (conjugate) for the antibody binding sites. After a wash step, substrate reacts with the bound enzyme conjugate to produce blue color. A stop solution is then add which changes the color from blue to yellow. The intensity of the color is inversely proportional to the concentration of ochratoxin A in the samples and standards.

Reagents And Materials Provided

1. Microtiter plate (8 wells×12) precoated with antibodies to mouse IgG.
2. Ochratoxin A standard solutions (1ml/each):0ng/mL, 0.2ng/mL, 0.8ng/mL, 3.0ng/mL, 10.0ng/ml
3. OTA Enzyme Conjugate :(10ml)
4. OTA Antibody : (10ml)
5. OTA Diluent Buffer: (50ml)
6. Washing buffer (10×) :(50ml)
7. TMB :(17ml)
8. Stop solution : (7ml)

Materials Required But Not Supplied

1) 20-200µl and 200-1000µl precision micropipette.
2) 50-300µl multichannel micropipette.
3) Microtitre plate reader with 450nm filter (Model MK-3Ex iElisa® Reader or equivalent)
4) Methanol - ACS grade or better.
5) Multi-purpose rotary mixer
6) 50mL centrifuge tube, funnel and paper filter

7) 100ml graduated cylinder.
8) vortex mixer
9) Timer

Storage

2-8°C (for sealed box), please do not freeze! See kit label for expiry date

Specimen Collection And Preparation

Corn and grain. The dilution factors are 10.

1. Transfer 5.0 grams of ground sample into a 50mL centrifuge tube
2. Add 25ml of the (70/30) methanol/water extraction solvent.
3. Cover the extraction jar and mix it with rotary mixer for 10 min.(or shake vigorously by hand for 3 min, or blend for 1min.)
4. Filter the extract through a paper filter.
5. Dilute 200µl of the filtrate with 200µl of OTA Diluent Buffer.

Feed. The dilution factors are 25.

1. Transfer 5.0grams of ground sample into a 50mL centrifuge tube
2. Add 25ml of the (70/30) methanol/water extraction solvent.
3. Cover the extraction jar and mix it with rotary mixer for 10 min.(or shake vigorously by hand for 3 min, or blend for 1min.)
4. Filter the extract through a paper filter.
5. Dilute 100µl of the filtrate with 400µl of OTA Diluent Buffer.

Urine. The dilution factor is 10.0

1. Centrifugation: Transfer 2.0 mL sample into a centrifugal screw cap vial, 10 min.4000r/min
2. Dilute 50uL of the supernatant with 450uL of OTA Diluent Buffer. Mix it well.
3. Use 50uL per well in the assay

Reagent Preparation

1. Ochratoxin A standard solutions: ready to use.
2. OTA Enzyme Conjugate: ready to use.
3. OTAAntibody: ready to use.
4. Washing buffer: dilute 10x with distilled water (1+9). (e.g. 10 ml buffer concentrate+90 ml distilled water, sufficient for 4 microtiter strips 32 wells).
5. TMB: ready to use.
6. Stop solution: ready to use.
7. 70 percent methanol/water: 700 ml +300 ml pure, or deionized or distilled water.

Assay Procedure

1. Allow reagents, microwells, and sample extracts to reach room temperature prior to running the test.
2. Insert a sufficient number of wells into the microwell holder for all standards and samples to be tested
3. Using a new pipette tip for each standard and sample, pipet 50µl of standards and prepared sample to separate wells.
4. Add 50µL of OTA Enzyme conjugate into each well
5. Add 50µL of OTA Antibody into each well.
6. Incubate for 40 minutes in room temperature (20-25°C).
7. Dump the contents of the wells. Turn the wells upside down and tap out on a paper towel until the remaining liquid has been removed.
8. Fill completely all the wells with working wash solution 250µL, empty them by inverting the plate, repeat 4 times. Finally, remove the residual droplets by vigorous knocking on absorbent laboratory towels.
9. Add 150µL of TMB to each well.
10. Incubate for 15 minutes in room temperature (20-25°C). Cover the wells with a paper towel to protect them from light sources.
11. Add 50µL ofstop solution to each well.
12. After thorough mixing the absorbance is measured using a microplate reader fitted with a 450 nm filter

Calculation

Divide the mean absorbance value of standards and samples (B) by the mean absorbance value of the Maximum Binding (B_0) and multiply by 100. Maximum binding is thus made equal to 100% and the absorbance values are quoted in percentages:

$$\frac{\text{absorbance standard(or sample)}}{\text{absorbance Maximum Binding}} \times 100 = \frac{B}{B_0}(\%)$$

Interpretation Of Results

Since the samples have been diluted prior to assay, the concentrations in ng/ml read from the standard curve must be multiplied by the respective dilution factor to obtain the effective ochratoxin A concentration in samples expressed in ppb (ng/ml or ng/g). The dilution factors are 10 for corn and grain. The dilution factors are 25 for feeds.

Precision

Intra-CV < 10%

Inter-CV < 15%

Sensitivity

0.2ppb

Specificity

OTA: 100%

OTB: 124%

OTC: 67%

Precautions

1. Each reagent is optimized for use in the ochratoxin A kit. Do not substitute regents from any other manufacturer into the test kit. Do not combine reagents from other kits with different Lot numbers.
2. The standard solutions contain ochratoxin A, particular care should be taken. Avoid contact of the reagent with the skin (use gloves).
3. Store the kit at 2 - 8 °C (35 - 46 °F). Do not freeze any test kit components. Return any unused microwells to their original foil bag and reseal them together with the desiccant provided at 2 - 8 °C (35 - 46 °F).
4. Do not use reagents after expiration date. 5) The TMB is light sensitive, therefore, avoid exposure to direct light.
6. Do not interchange individual reagents between kits of different lot numbers