Ochratoxin A ELISA Kit

Enzyme Immunoassay for the quantification determination of Ochratoxin in food

Catalog number: ARG83049

Package: 96 wells

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INTRODUCTION

Ochratoxin A (OTA) is a naturally occurring foodborne mycotoxin found in a wide variety of agricultural commodities worldwide, ranging from cereal grains to dried fruits to wine and coffee. It is produced by several different fungi including Aspergillus ochraceus, A. carbonarius, A. niger and Penicillium verrucosum. These fungi vary in their optimal growing temperatures and water activity, and contaminate various commodities. Contamination generally occurs as a result of poor storage of commodities and suboptimal agricultural practices during the drying of foods.

OTA is a chemically stable compound; hence, ordinary food processing measures fail to substantially reduce its presence in foods and beverages. OTA has been shown to be toxic and carcinogenic in animals. The kidney is the main target organ for OTA; OTA is a potent renal carcinogen in several animal species . Other adverse effects of OTA include immunotoxicity , inhibition of macromolecular synthesis, increased lipid peroxidation, and inhibition of mitochondrial respiration. OTA has been suspected as a cause of various human nephropathies since the 1970s including Balkan Endemic Nephropathy (BEN) . The International Agency for Research on Cancer (IARC) has classified OTA as a Group 2B possible human carcinogen, based on demonstrated carcinogenicity in animal studies, although OTA-related carcinogenicity has not been conclusively determined in humans. A recent risk assessment on OTA states that OTA was negative in genotoxicity assays with high specificity, and that OTA-DNA adduct levels were low and not typical of genotoxic carcinogens.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative competitive enzyme immunoassay technique. Ochratoxin A antibody is bind on the surface of a microtiter plate. Ochratoxin A containing samples or standards, Ochratoxin A-Peroxidase and an antibody directed against Ochratoxin A are given into the wells of the microtiter plate. Immobilized and free Ochratoxin A compete for the antibody binding sites. After 10 minutes incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. Then a substrate solution is added and incubated for 5 minutes, resulting in the evelopment of a blue color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The yellow color is measured at 450 nm. The concentration of Ochratoxin A is indirectly proportional to the color intensity of the test sample.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard A-F (0, 2, 5, 10, 20, 50 ppb)	6 X 1 ml (Ready-to-use)	4°C.
Ochratoxin A Antibody	6 ml (Ready-to-use)	4°C
HRP-Ochratoxin A Conjugate	6 ml (Ready-to-use)	4°C
10X Wash buffer	60 ml	4°C
TMB substrate	15 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	15 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C in the dark at all times. Do not expose reagents to heat, sun, or strong light during storage and usage.
- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days

when stored at 2-8°C. We recommend consumption on the same day.

- Briefly spin down the antibody conjugate Mixture and HRP-Streptavidin concentrate before use.
- All materials must be at room temperature (20-28°C) prior to use.
- If crystals are observed in the 20X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- All incubation steps must be accurately timed.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

Due to high risk of cross-contamination all applied instruments like applicator, mortar, glass vials etc. have to be cleaned thoroughly before and after each sample. Some proteins adhere very strongly to different surfaces. In certain cases they can resist a common dishwasher cleaning. To identify possible cross-contamination caused by previous extractions it is strongly recommended to note the sequence of the extractions.

Cereals- Grind sample to pass through sieve and suspend 20 g of sample in 50 mL of 70% methanol and centrifuge at 3000 g for 5 minutes. Dilute equal volume of supernatant of distilled water. If clouds appear during final dilution with distilled water, it is recommend to centrifuge at 3000 g for 5 minutes another time and test the supernatant.

Beer- Beer samples should be preliminary degassed by moderate heating. The cloudy beers should preliminarily be sterile-filtered. User can dilute beer with 35% methanol.

Wine- Adjust the pH to 6.5. Add 0.5 g of extraction additive (DEEXSCH2) to 10 mL of sample (pH 7.0) and mix suspension for 3 min, then centrifuge at 3000 g for 5 minutes. User can dilute with 40% methanol.

Other samples- Grind sample to pass through sieve and suspend 20 g of sample in 50 mL of 70% methanol and centrifuge at 3000 g for 5 minutes. Dilute equal volume of supernatant of distilled water. If clouds appear during final dilution with distilled water, it is recommend to centrifuge at 3000 g for 5 minutes another time and test the supernatant.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 10 ml of 10X Wash buffer + 90 ml of distilled water) The diluted Wash buffer is stable for 30 days at 2°C to 8°C.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-28 °C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **50 µl** of **standards and prepared samples** into wells.
3. Add **50 µl** of **HRP-Ochratoxin A Conjugate** into wells.
4. Add **50 µl** of **Ochratoxin A Antibody** into wells.
5. Incubate for **10 minutes at RT**.
6. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes**. Wash by filling each well with **1× Wash Buffer (300µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
7. Add **100 µl** of **TMB Reagent** to each well. Incubate for **5-7 minutes at room temperature in dark**.
8. Add **100 µl** of **Stop Solution** to each well. The color of the solution should change from blue to yellow.
9. Read the OD with a microplate reader at **450 nm** immediately (optional: Read the OD at 600-690nm as reference). It is recommended read the absorbance within 30 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

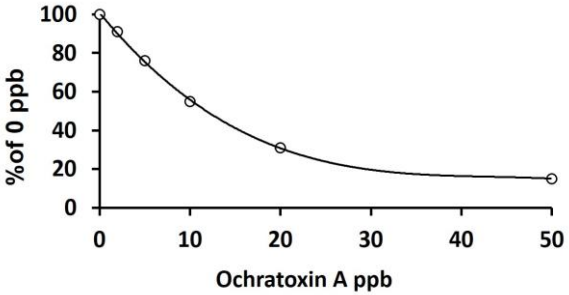
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. If these quality control criteria are not met the assay run is invalid and should be repeated.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The standard range of Ochratoxin A ranged from 2-50 ppb. The mean Limit of detection was 0.8 ppb.

Cross-reactivity

7 % Ochratoxin B (Relative to Ochratoxin A (=100%)).

Linearity

90%- 117%

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 4.6% and inter-assay precision was 9.7%.