BIOEASY

Immunoaffinity Column for Ochratoxin A(OTA)

Order code: YRIAC3005-1C

Introduction

Ochratoxin is a class of mycotoxin mainly produced by certain strains of Aspergillus ochraceus, Aspergillus niger, and Penicillin. It is one of the five categories of mycotoxins currently receiving widespread attention, with seven structural similar compounds. Ochratoxin A (OTA) is the most toxic, most widely distributed, and most closely related to human health among them, and also has the highest pollution levels and detection rate among substrates such as crops.

OTA is sensitive to light and air, relatively stable to heat, and can still live through normal processing. The main target organ is the kidney, which has strong nephrotoxicity and potentially carcinogenic, teratogenic, and mutagenic hazards. Therefore, the International Agency for Research on Cancer (IARC) defines it as a Class B (ⅡB) carcinogen. OTA is second only to AFs in terms of the importance and harmfulness of mycotoxins that have been discovered.

Principle

Based on the specific binding of antibody and antigen, the monoclonal antibodies of OTA were fixed in the column to make an immunoaffinity column.

After the sample is extracted, centrifuged or filtered, and the supernatant is appropriately diluted, the OTA in the sample slowly passes through the immunoaffinity column and specifically binds to the antibody. Rinse the immunoaffinity column to remove other substances that are not bound and elute OTA with methanol. It can be used for analytical instrument detection after proper dilution.

Application

This product is suitable for the pre-processing of samples containing OTA.

After the sample solution is purified by the immunoaffinity column, it can be directly used for qualitative and quantitative detection by HPLC, LC-MS, and other analytical instruments. It can effectively improve the signal-to-noise ratio and increase the sensitivity and accuracy of the detection method.

Solid Sample

Grain: corn, wheat, wheat flour, brown rice, rice, barley, etc.

Oil crops and seeds: soybean, rapeseed

Oiled chili products: chili, black pepper

Coffee: ground coffee, instant coffee, coffee beans

Others: raisins

Liquid Sample

Vegetable oils: sesame oil, corn oil, peanut oil, blended oil, etc.

Condiments: soy sauce, white vinegar, mature vinegar

Alcohol: Chinese spirits, beer, wine

Semi-liquid sample

Soybean paste, etc.

【Note: Please select the extraction method according to the type of sample for samples not listed in detail. If in doubt, please contact the product manager or send samples to Bioeasy for confirmation method.】

Performance Information

Column capacity: 300ng

Column recovery rate: ≥80%

Storage and Shelf Life

Storage: Store at 2-8℃(Don't be close to the inner wall of the refrigerator.) Do not freeze. Keep away from direct sunlight, moisture and heat.

Shelf Life: 12 months.

Components (25 PCs/Kit)

1. 25 PCs of 3mL Immunoaffinity Column
2. 1 instruction manual

Materials Required but not Provided (Available from BIOEASY)

Equipment:

1. Homogenizer, high-speed pulverizer, tissue pulverizer

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2. Sieve: 1mm-2mm aperture test sieve
3. Balance: 0.01 g sensibility
4. Vortex mixer
5. Ultrasonic or vortex shaker
6. High-speed homogenizer: 6500 r/min-24000r/min
7. Centrifuge: speed⩾6000 r/min
8. Solid Phase Extraction device (with a vacuum pump)
9. Termovap Sample Concentrator
10. Analytical instruments such as Liquid Chromatography and Liquid Chromatograph-Mass Spectrometer, etc.
11. 50mL graduated cylinder
12. Single channel pipette: 10 μL-100 μL, 100 μL-1000 μL, 1000 μL-5000 μL
13. pH meter (or pH test strip)

Consumables:

1. Centrifuge tube (4mL, 50mL)
2. Syringe or loading tube (30mL)
3. Glass microfiber filters: required fast, high load, particle retained in liquid is 1.6 μm
4. 1mL Disposable syringe
5. Disposable millipore filter head with 0.22 μm millipore filter membrane (Test the selected filter membrane with the standard solution to confirm no adsorption phenomenon before it can be use).

Reagents

(Unless otherwise specified, all reagents used are of analytical grade, and the water is pure water.)

1. Methanol: chromatographic purity
2. Acetonitrile: chromatographic purity
3. Sodium chloride
4. 1M sodium hydroxide: Weigh 4.00g sodium hydroxide, dissolve in water, and dilute to 100mL.
5. 1M hydrochloric acid: Take 8.5mL hydrochloric acid, dilute to 91.5mL with water, and mix well.
6. Phosphate Buffer Solution (PBS) with pH 7.4: Weigh 8.00g sodium chloride, 1.20g disodium hydrogen phosphate, 0.20g potassium dihydrogen phosphate, and 0.20g potassium chloride, dissolve in water and dilute to 1000mL.
7. 1% Tween-20 in PBS (PBST): Take 10mL Tween-20 and dilute to 1000mL with PBS.
8. Methanol water-solution (80+20): Take 800mL methanol, add 200mL water, and mix well.

Sample Preparation

Solid samples

Use a high-speed pulverizer to pulverize sample, sieving to make the particle size smaller than 2mm aperture, mix evenly and subpackage to 100g in the sample bottle, sealed and preserved for testing.

Liquid samples

Mixing well all the liquid samples in a container by the homogenizer, take 100g (mL) sample to be tested.

Semi-liquid sample

Mashing and mixing with a tissue masher, sealed in the sample bottle and preserved for testing.

Sample Extraction

- **Solid samples**

  Grain: corn, wheat, wheat flour, brown rice, rice, barley, etc.

1. Weigh 5.00 g sample into the 50 mL centrifuge tube.
2. Add 20mL of methanol water-solution (80+20), vortex to mix, and shake in an ultrasonic/vortex shaker for 30 mins (or homogenize with the homogenizer for 3 mins).
3. Centrifuge for 10 mins at ⩾6000 r/min (or filter with glass fiber filter paper after homogenization, except for grease) to obtain the supernatant for preparation of sample solution.

- **Solid samples**

  Oil crops and seeds: soybean, rapeseed

  Oiled chili products: chili, black pepper

  Coffee: ground coffee, instant coffee, coffee beans

  Others: raisins

  Liquid samples

  Vegetable oils: sesame oil, corn oil, peanut oil, blended oil, etc.

  Semi-liquid sample

Soybean paste, etc.

1. Weigh 5.00 g sample into the 50 mL centrifuge tube.
2. Add 1.00 g NaCl.
3. Add 20mL of methanol water-solution (80+20), vortex to mix, and shake in an ultrasonic/vortex shaker for 30 mins (or homogenize with the homogenizer for 3 mins).
4. Centrifuge for 10 mins at ⩾6000 r/min (or filter with glass fiber filter paper after homogenization, except for grease) to obtain the supernatant for preparation of sample solution.

- **Liquid Sample**

Alcohol: Chinese spirits, beer, wine

【Note: Alcohol samples need to be degassed first.】

1. Weigh 5.00 g sample into the 50 mL centrifuge tube.
2. Add 20mL of PBS, vortex to mix.
3. Centrifuge for 10 mins at ⩾6000 r/min (or filter with glass fiber filter paper after homogenization, except for grease) to obtain the supernatant for preparation of sample solution.

- **Liquid Sample**

Condiments: soy sauce, white vinegar, mature vinegar

1. Weigh 10.00 g sample into the 50 mL centrifuge tube.
2. Add 10mL of methanol water-solution (80+20), vortex to mix.
3. Centrifuge for 10 mins at ⩾6000 r/min (or filter with glass fiber filter paper after homogenization, except for grease) to obtain the supernatant for preparation of sample solution.

Sample Solution Preparation

- **Solid samples**

Grain: corn, wheat, wheat flour, brown rice, rice, barley, etc.

Oil crops and seeds: soybean, rapeseed

Oiled chili products: chili, black pepper

Coffee: ground coffee, instant coffee, coffee beans

Others: raisins

Liquid samples

Vegetable oils: sesame oil, corn oil, peanut oil, blended oil, etc.

Semi-liquid sample

Soybean paste, etc.

1. Accurately pipette 5mL supernatant (equivalent to 1g sample).
2. Add 20mL PBS and mix well.
3. Centrifuge at ⩾6000 r/min for 10 mins to obtain 20mL supernatant as sample solution.

- **Liquid Sample**

Condiments: soy sauce, white vinegar, mature vinegar

1. Accurately pipette 5mL supernatant (equivalent to 2.5g sample).
2. Add 20mL PBS and mix well.
3. Centrifuge at ⩾6000 r/min for 10 mins to obtain 10mL supernatant as sample solution.

【Note: When the sample is such as vinegar, please use 1M sodium hydroxide to adjust the pH of the sample solution to 7.4】

- **Liquid Sample**

Alcohol: Chinese spirits, beer, wine

Accurately pipette 10mL supernatant as sample solution.

Sample Solution Purification

1. Return the immunoaffinity column to room temperature before use, connect the syringe barrel or loading tube to the immunoaffinity column, and completely dripped out the original liquid in the column.
2. Accurately pipette the sample solution into the syringe barrel or loading tube, and the sample solution drip at a rate of 1-2 drops per second under gravity pressure.
3. The rinsing procedure for different samples are as follows

- **Solid samples**

Grain: corn, wheat, wheat flour, brown rice, rice, etc.

Oil crops and seeds: rapeseed

Oiled chili products: chili, black pepper

Liquid samples

Vegetable oils: sesame oil, corn oil, peanut oil, blended oil, etc.

Alcohol: Chinese spirits, beer, wine

1) After the sample solution drop is finished, add 20mL PBS to the syringe barrel or loading tube to rinse the immunoaffinity column.
2) Use a vacuum pump to drain the immunoaffinity column after the drop is finished.

- **Solid samples**

Grain: barely

Oil crops and seeds: soybean

Coffee: ground coffee, instant coffee, coffee beans

Others: raisins

- **Liquid Sample**

Condiments: soy sauce, white vinegar, mature vinegar

- **Semi-liquid sample**

Soybean paste, etc.

1) After the sample solution drop is finished, add 20mL PBST to the syringe barrel or loading tube to rinse the immunoaffinity column.
2) Use a vacuum pump to drain the immunoaffinity column after the drop is finished.

4. Remove the syringe barrel or loading tube, place a 4mL centrifuge tube under the immunoaffinity column.
5. Add 2mL methanol to elute the immunoaffinity column. Use a vacuum pump to drain the immunoaffinity column after the drop is finished. If there is no liquid dripping out after about 1min, please use a syringe to give a little pressure to make the 1- 2 drops of liquid be dripped out, continue to drip out under gravity pressure.
6. Collect all the eluates and mix well.
7. After the eluent is blown to near dryness with nitrogen, reconstitute with 1mL initial mobile phase of HPLC or LS-MC, filter with 0.22 μm microporous filter, and transfer to sample bottle for testing.

Result Interpretation

The content of OTA in 2mL of eluent is equivalent to the content of OTA in 1g of the sample (Condiments sample is 2.5g, Alcohol sample is 2g)

OTA content = Detection Concentration × Dilution Factor

Precautions

1. The entire analysis operation should be carried out in the required area. The area should be a relatively independent operating table and waste storage device, and keep away from direct sunlight.
2. During the entire experiment, the operator should take corresponding protective measures in accordance with the requirements for exposure to highly toxic substances.
3. Take out the required number of immunoaffinity columns before use and return them to room temperature.
4. Do not use the immunoaffinity column after the expiration date.
5. The amount of sample to be weighed can be appropriately increased or decreased according to needs, and the amount of sodium chloride and extracting solution can be increased or decreased proportionally.
6. When the content of the toxin in the sample divided by the dilution factor is higher than the column capacity, it is necessary to retest. The customers can appropriately reduce the volume of the sample solution or increase the dilution factor.
7. The optimum pH of the sample solution is between 7-8, check the pH by the pH meter (or pH test paper) before dropping the sample solution into the column. If the pH isn't within this range, adjust the pH with sodium hydroxide or hydrochloric acid.
8. It is recommended to soak the used container and mycotoxin solution with 5% sodium hypochlorite solution (V/V) overnight.

Validation of Column Capacity and Column Recovery

1. In 60mL of 16% methanol-PBS, add 900ng OTA standard stock solution and mix well to obtain sample solution.
2. Take three immunoaffinity columns from the same batch, pipette 20mL sample solution in step 1 (equivalent to 300ng OTA) into each immunoaffinity column.
3. The rinse and elution procedure are the same as "Sample Solution Purification". Rinse with PBS.
4. Detection and analysis.

The result interpretation:

The result of OTA is≥240ng show that the recovery rate is ≥80%. The product is valid.

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