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MaxSignal© IAC for Ochratoxin A

Ochratoxin A Immunoaffinity Column

Catalog #FOOD-1511-01

ISO 9001 QUALITY ASSURANCE

Manufactured in compliance with our ISO 9001 certified quality management system.

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RELATED PRODUCTS

CATALOG #	PRODUCT	QTY
FOOD-1502-01	MaxSignal® IAC 4-in-1 Combo for Aflatoxin B_1, Zearalenone, Vomitoxin, and Ochratoxin A	25 Tests
FOOD-1503-01	MaxSignal® IAC 2-in-1 Combo for Total Aflatoxin and Ochratoxin A	25 Tests
FOOD-1505-01	MaxSignal® IAC 6-in-1 Combo Mycotoxin Immunoaffinity Column	10 Tests

GENERAL INFORMATION

Purpose

The immunoaffinity column can selectively adsorb Ochratoxin A from the sample solution, thereby having a highly targeted purification effect on the sample. The sample solution that has been purified by passing through the column can be directly used for HPLC analysis or for LC-MS/MS analysis after being concentrated with nitrogen gas and resuspended.

Affinity columns can be used in combination with HPLC/LC-MS/MS to achieve rapid testing, and to increase signal-to-noise ratio and improve the accuracy of the detection method.

Overview

Ochratoxin A (OTA) is a toxic metabolite produced by some species of Aspergillus and Penicillium; it is a mycotoxin with high kidney and liver toxicities, and has teratogenic, mutagenic, and carcinogenic effects.

Principle

This IAC functions by an antigen-antibody interaction. The main toxin antibody is embedded throughout the column. After a sample is extracted and filtered, it is slowly passed through the IAC. The toxoids bind to their corresponding antibody in the column. The IAC is then washed to remove unbound substances. The toxins are then eluted with the eluent, concentrated using nitrogen gas, then injected into an analytical instrument for detection.

KIT CONTENTS, STORAGE, & SHELF LIFE

Each box contains Ochratoxin A immunoaffinity columns of various specifications and 1 instruction manual. Store the entire kit at 2–8°C. Do not use this product past the expiration date indicated on the box label.

Required Materials Not Provided with the Kit

- HPLC
- Centrifuge capable of at least 3,000-4,000 x g
- Nitrogen gas evaporator apparatus
- Nitrogen gas tank and pressure regulator
- LC-MS (LC-MS/MS)
- Air-pressure controller
- Air pump
- Balance with 0.01 g readability
- High-speed homogenizer (i.e. rotary shaker, vortexer, stomacher, or equivalent) (maximum speed ≥ 10,000 RPM)
- Grinder
- Sieving screen: 2-mm
- pH meter (or pH test paper)
- Graduated cylinder: 10 mL & 100 mL
- Funnel: 50 mL
- Syringe: 10 mL & 20 mL
- Pipette and pipette tips
- Homogenization flask (or 250-mL conical flask with pestle)
- Sample tubes and bottles
- Qualitative filter paper
- Microfiber filter paper (e.g. Whatman 934-AH)
- Column holder and syringe connector plug (for use with 6-mL immunoaffinity columns)
- Methanol (CH_3OH): Chromatography Grade
- Acetonitrile (CH_3CN): Analytical Grade
- Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$): Analytical Grade
- Acetic acid (CH_3COOH): Chromatography Grade
- Potassium dihydrogen phosphate (KH2PO4): Analytical Grade
- Potassium chloride (KCl): Analytical Grade
- Sodium chloride (NaCl): Analytical Grade
- Tween-20® ($C_{58}H_{114}O_{26}$): Analytical Grade
- Polyethylene glycol: Analytical Grade; polyethylene glycol is used to improve the filtration speed, if the supernatant is obtained by centrifugation, this reagent can be omitted
- Hydrochloric acid (HCl): Analytical Grade
- Sodium hydroxide (NaOH): Analytical Grade
- Distilled/deionized water

PRECAUTIONS

- Allow the immunoaffinity column to equilibrate to room temperature (20–25°C) before use.
- The immunoaffinity column should be stored at 2–8°C; do not freeze.
- Do not use any expired immunoaffinity column.
- The sample volume can be increased or decreased appropriately as needed. The volume of the extraction solution should be adjusted accordingly.
- The pH of the loading solution onto the immunoaffinity column should be 6–8. If it deviates from this range, the pH should be adjusted with dilute hydrochloric acid or dilute sodium hydroxide.
- Maintaining consistency (such as polarity, pH, and concentration) between the test solvent loaded into any analytical instrument and the mobile phase can help eliminate any adverse solvent effects.
- Column capacity: the column capacity is 100 ng, when the content of the toxin in the sample divided by the dilution factor is higher than the column capacity, it is necessary to reduce the volume of the sample solution appropriately, and retest.
- WARNING: Ochratoxins are carcinogenic; protective equipment such as gloves and masks should always be used during handling.
- Vessels and tools used to handle toxin solutions should be completely immersed in a sodium hypochlorite solution (5% v/v) overnight.
- Ensure the LC-MS/MS is clean and the tubing is primed appropriately for each run.
- Follow appropriate instrument precautions if using HPLC.

REAGENT PREPARATION

1. **Preparation of Extraction Liquid 1 (for the extraction of grain samples and soy sauce and vinegar samples)**
   Combine 800 mL of methanol and 200 mL of distilled/deionized water. Bring to 1 L final volume with distilled/deionized water. Mix well.
2. **Preparation of Extraction Liquid 2 (For the extraction of alcoholic samples)**
   Combine out 150 g of sodium chloride and 20 g of sodium bicarbonate and dissolve in approximately 950 mL of distilled/deionizedwater, mix, then bring to a final volume of 1 L with distilled/deionized water.
3. **Preparation of Wash Solution (For washing of soy sauce and vinegar samples)**
   Combine 12.50 g of sodium chloride and 2.5 g of sodium bicarbonate and dissolve in water, add 0.1 ml of Tween-20 and bring to a final volume of 1 L with distilled/deionized water.
4. **Preparation of Wash Liquid (for washing in the purification step for alcoholic samples)**
   Combine 25 g of sodium chloride and 5 g of sodium bicarbonate and dissolve in approximately 950 mL of water, mix, then bring to a final volume of 1 L with distilled/deionized. and top up the volume with water to 1 L.
5. **Preparation of Eluent Solution (for elution of all samples after they pass through the column) 2% v/v acetic acid-methanol**
   Combine 2 mL of acetic acid and 98 mL of methanol. Mix well.

SAMPLE PREPARATION

Method 1: Grains

1. Add 20 g ± 0.01 g of sample (solid samples must be pulverized and passed through a 2-mm sieving screen) and 5 g of sodium chloride into a conical flask, then add 100 mL of Extraction Liquid 1.
2. Homogenize, such as vortex, at high speed (≥ 10,000 RPM) for 1 minute, or shake vigorously on a shaker (200-300 RPM) for 20 minutes.
3. Filter with rapid qualitative filter paper, and collect the filtrate.
4. Combine 10 mL of filtrate with 40 mL of distilled/deionized water to dilute, mix well.
5. Filter with microfiber filter paper. Collect the filtrate.
6. Use 25 mL of the sample with the immuoaffinity column for purification.

Dilution factor = 1

Method 2: Alcohols

1. Take 20 g ± 0.01 g of a degassed alcohol sample (for alcohol samples containing carbon dioxide, stir or degas using ultrasound before use) or an alcohol sample without carbon dioxide, add Extraction Liquid 2 and bring to volume of 25 mL.
2. Homogenize, such as vortex, at high speed (≥ 10,000 RPM) for 1 minute, or shake vigorously on a shaker (200-300 RPM) for 20 minutes.
3. Filter with microfiber filter paper. Collect the filtrate.
4. Use 1 mL of the sample with the immuoaffinity column for purification.

Dilution factor = 1.25

Method 3: Soy sauce and vinegar samples

1. Take 25 g ± 0.01 g of sample, add Extraction Liquid 1 and bring to volume of 50 mL.
2. Homogenize, such as vortex, at high speed (≥ 10,000 RPM) for 1 minute, or shake vigorously on a shaker (200-300 RPM) for 20 minutes.
3. Use 25 mL of the sample with the immunoaffinity column for purification.

Dilution factor = 0.4

OPERATING PROCEDURE

1. Remove the column and place into a column holder. Remove the plunger of a syringe, then attach the syringe through the connector plug above the column to complete the connection. Secure to an air-pressure controller, if available.
2. Transfer the appropriate amount of the solution processed in Sample Preparation to fill the syringe.
3. Remove the cap under the affinity column (do not discard as this will be used in the next step). Adjust the air-pressure to have a flow rate of 1–2 drops/second.
4. If the sample to be purified is a grain, after the liquid has completely flowed through, add 10 mL of distilled/deionized water at a flow rate of 2-3 drops per second.
5. If the sample to be purified is soy sauce or vinegar, after the liquid has completely flowed through, add 10 mL of Wash Solution at a flow rate of 2-3 drops per second.
6. If the sample to be purified is an alcohol, after the liquid has completely flowed through, add 10 mL of Washing LIquid, then 10 mL of water at a rate of 2-3 drops per second.
7. After the liquid has completely flowed through replace the syringe with a new one, add 2 mL of eluent, use a test tube to collect the eluent at a flow rate of 1 drop per second, collect the eluent and bring to volume of 2 mL.
8. Filter the eluent through a 0.22 µm micropore filter and then transfer into a sample bottle to be used for HPLC analysis.

** Also applies to GB 5009.96-2016 and GB/T 30957-2014.*

INTERPRETATION OF RESULTS

Ochratoxin A Concentration = Detected Concentration x Dilution Factor x 2