PerkinElmer®
For the Better

MaxSignal® IAC 4-in-1 Combo

Immunoaffinity Column for Aflatoxin B_1, Zearalenone, Vomitoxin, and Ochratoxin A

Catalog #FOOD-1502-01

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-1501-01	MaxSignal® IAC 4-in-1 Combo for Aflatoxin B_1, Zearalenone, Fumonisin, Vomitoxin and its derivatives	25 tests
FOOD-1503-01	MaxSignal® IAC 2-in-1 Combo for Total Aflatoxin and Ochratoxin A	25 tests
FOOD-1504-01	MaxSignal® IAC 3-in-1 Combo for Total Aflatoxin, Zearalenone, and Vomitoxin	25 tests
FOOD-1505-01	MaxSignal® IAC 6-in-1 Combo Mycotoxin Immunoaffinity Column	10 tests
FOOD-1507-01	MaxSignal® IAC for Aflatoxin B_1	25 tests

GENERAL INFORMATION

Purpose

The immunoaffinity column can simultaneously extract Aflatoxin B_1 (AFT B_1), Zearalenone (ZEN), Vomitoxin (DON), Ochratoxin A (OTA) from the sample extraction solution, and thereby have a highly targeted purification effect on these four types of toxins. The samples that have been passed through the column, blown-dried with nitrogen and redissolved in a suitable solvent, can be directly used for LC-MS (LC-MS/MS) analysis to determine the content of these four types of toxins.

Overview

Aflatoxins are toxic metabolites of a class of fungi (such as Aspergillus flavus and Aspergillus parasiticus). They are highly carcinogenic. Zearalenone (ZEN), also known as F-2 toxin, mainly pollutes crops such as corn, wheat, and grains. It has a strong estrogenic effect and can cause hyperestrogen, as well as severe reproductive tract symptoms and infertility, and is also immunotoxic and genotoxic. Deoxynivalenol (DON) also known as Vomitoxin, belongs to the thichothecene family. Due to its cytotoxicity and immunosuppressive properties, it poses a health threat to both humans and animals. Ochratoxin A is produced by a variety of Aspergillus and Penicillium growing on crops such as food, peanuts, beans, etc. Ochratoxins mainly have detrimental effects on the liver and kidneys of animals, hence causing liver damage, and have teratogenic effects.

Principle

This IAC functions by an antigen-antibody interaction. All four main toxin antibodies are embedded throughout the column. After a sample is extracted and filtered, it is slowly passed through the IAC. The toxins 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 AFT B_1, ZEN, DON, and OTA 4-in-1 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

- 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 (KH_2PO_4): Analytical Grade
- Potassium chloride (KCl): Analytical Grade
- Sodium chloride (NaCl): Analytical Grade
- Tween-20® ($C_{58}H_{114}O_{26}$): Analytical Grade
- 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 injected into any analytical instrument and the mobile phase can help eliminate any adverse solvent effects.
- Column capacity:

Toxin name	Column capacity: ng
Aflatoxin B_1	300
Zearalenone	2000
Vomitoxin	2000
Ochratoxin A	100

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 test again.

- WARNING: Aflatoxin B_1, zearalenone, ochratoxin A, and vomitoxin are toxic and 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 Solution: 80% v/v Acetonitrile-water**
   Combine 800 mL of acetonitrile and 200 mL of distilled/deionized water. Bring to 1 L final volume with distilled/deionized water. Mix well.
2. **Preparation of Diluent Solution: 0.05M PBS, pH 7.3**
   Weigh out 8 g of NaCl, 0.2 g of KCl, 0.2 g of KH2PO4 and 1.16 g of Na2HPO4·12H2O into a large, graduated bottle. Dissolve with 800 mL of distilled/deionized water, then bring to a final volume of 1 L. Mix well.
3. **Preparation of Wash Solution: 0.1% v/v Tween-20 aqueous solution**
   Combine 1 mL of Tween-20 and 999 mL of distilled/deionized water. Mix well.
4. **Preparation of Eluent Solution: 2% v/v acetic acid-methanol**
   Combine 2 mL of acetic acid and 98 mL of methanol. Mix well.

SAMPLE PREPARATION

1. Weigh 25 g ± 0.01 g of sample into a bottle. Add 125 mL of Extraction Solution. Solid samples should be homogenized to pass through a 2-mm sieve.
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 or centrifuge at 3,000-4,000 x g for 5 minutes.
4. Combine 10 mL of filtrate (or the supernatant after centrifugation), with 70 mL of diluent. Mix well.
5. Filter with microfiber filter paper until the solution is clear
6. Use 20 mL of the filtrate (equivalent to 0.5 g of the sample) as the final sample for testing.

Dilution Factor = 2

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. After the liquid has flowed through, add 10 mL of Wash Solution. Allow the liquid to fow through, then add 10 mL of water at a flow rate of 2–3 drops/second.
5. After the liquid has flowed through and with the column plug attached, add 2 mL of Eluent Solution. Incubate for 3 minutes, then elute at a rate of 1 drop per second, and collect the eluate.
6. Place the eluent under a slow stream of nitrogen gas at 50°C to evaporate any residual solvents. Dissolve the dried residue with 1 mL of the approriate solvent. Filter the solution through a 0.22 µm micropore filter, then transfer to a sample bottle to be used for LC-MS (LC-MS/MS) analysis.

INTERPRETATION OF RESULTS

Toxin Content = Detected Concentration x Dilution Factor

NOTES