PerkinElmer®
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MaxSignal® IAC 4-in-1 Combo

Immunoaffinity Columns for Aflatoxin B_1, Zearalenone, Vomitoxin and its derivatives, and Fumonisin

Catalog #FOOD-1501-01

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-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

This immunoaffinity column (IAC) can simultaneously adsorb AflatoxinB_1 (AFT B_1), Zearalenone (ZEN), Deoxynivalenol (DON) and its derivative 3-3-acetyldeoxynivalenol (3-ADON), 15-3-Acetyldeoxynivalenol (15-ADON), and Fumonisin (FB_1, FB_2, FB_3), and has a highly targeted purification effect on the above four main types of toxins from various sample types. Samples that pass through the column for purification can be used for LC-MS (LC-MS/MS) analysis after concentrating with nitrogen gas.

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 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 kit contains an all-in-one immunoaffinity column 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 loaded into any analytical instrument and the mobile phase can help eliminate any adverse solvent effects.
- Column capacity:

Toxin Name	Column Capacity: ng	Toxin Name	Column Capacity: ng
AFT B_1	300	ZEN	1000
DON	2000	FB_1	
3-ADON	1000	FB_2	5000
15-ADON	1000	FB_3	

- WARNING: Aflatoxin B_1, zearalenone, vomitoxin and its derivatives, and fumonisin are all toxic and carcinogenic; protective equipment such as gloves and masks should always be worn 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 1: 80% v/v Acetonitrile-water (containing 1% v/v acetic acid)**
   Combine 800 mL of acetonitrile and 10 mL of acetic acid in a graduated cylinder, then bring to 1 L 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 KH_2PO_4 and 1.16 g of $Na_2HPO_4 \cdot 12H_2O$ 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 100 mL of Extraction Solution 1. Solid samples should be homogenized to pass through a 2-mm sieve before use..
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. Centrifuge at 3,000-4,000 x g for 5 minutes.
4. Transfer 5 mL of supernatant to70 mL of Diluent Solution. Mix well.
5. Filter with microfiber filter paper.
6. Use 30 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 all the liquid has flowed through, add 10 mL of water to wash the column at a flow rate of 2–3 drops per second. Repeat this wash step one more time. Note: if the column appears darker due to the material passed through, pre-wash one time with 10 mL of Wash Solution before washing with water.
5. After the liquid has flowed through, load 2 mL of Eluent Solution. Cap the opening under the column using the plug, allow the column to incubate for 3 minutes. Place a collection tube under the column. After 3 minutes, remove the plug and allow the liquid to flow through at a rate of 1 drop per second. Collect this liquid known as the eluate.
6. After the liquid has flowed through, add another 1 mL of the Eluent Solution, cap the opening under the column using the plug, allow the column to incubate for 3 minutes. Place a collection tube under the column. After 3 minutes, remove the plug and allow the liquid to flow through at a rate of 1 drop per second. Combine this liquid with the eluate from step 5.
7. Place the eluate under a slow stream of nitrogen gas at 50°C to evaporate any residual solvents. Dissolve the dried residue with 1 mL of the appropriate solvent needed for downstream processing.
8. Inject 20 µL into LC-MS/MS for detection and analysis.

INTERPRETATION OF RESULTS

Toxoin Concentration = Detected Concentration x Dilution Factor

NOTES

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