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MaxSignal© IAC for Fumonisin

Fumonison Immunoaffinity Column

Catalog #FOOD-1510-01

ISO 9001 QUALITY ASSURANCE

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

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA

P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

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-1505-01	MaxSignal® IAC 6-in-1 Combo Mycotoxin Immunoaffinity Column	10 Tests

GENERAL INFORMATION

Purpose

The immunoaffinity column can selectively adsorb fumonisin (FB_1, FB_2, FB_3) 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 LC-MS/MS analysis after concentrating with nitrogen gas and resuspended or for HPLC analysis after derivatization.

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

Overview

Fumonisin is a metabolite produced by *Fusarium* fungi. It has acute and chronic toxicities, and is species-specific and organ-specific. Studies have found that high concentrations of fumonisin can cause acute species-specific toxicity symptoms in many domestic and laboratory animals, such as equine leukoencephalomalacia, porcine pulmonary edema, and liver and kidney diseases in sheep. The toxin has also been found to be related to esophageal and liver cancer in humans.

Principle

This IAC functions by an antigen-antibody interaction. All the 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 fumonisin 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:

Toxin name	Column capacity: ng
FB1	
FB2	5000
FB3	

- 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: Fumonisin is toxic; 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 (50% acetonitrile-water containing 1% acetic acid)**
   Combine 500 mL of acetonitrile and 10 ml of acetic acid, and add 490 mL of distilled/deionizedwater.
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 $Na_2HPO_4{\cdot}12H_2O$ 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 0.1 mL of Tween-20 and distilled/deionized water to total volume of 100 mL. 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.
5. **Preparation of Resuspension Solution**
   Pipette 50 mL of acetonitrile and add 50 mL of distilled/deionized water. Mix well.

SAMPLE PREPARATION

1. Weigh 25 g ± 0.01 g of the sample (solid samples should be ground and passed through a 2-mm sieving screen) and place into a container, add 100 mL of the Extraction Solution (50% acetonitrile-water containing 1% acetic acid).
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 1.5 mL of filtrate to a new tube and add 36 ml of diluent (0.1% Tween-20/PBS solution), shake to mix well;
5. Filter again with microfiber filter paper and collect the filtrate.
6. Use 20 mL of the filtrate (equivalent to 0.2 g of sample) with the immunoaffinity column for purification.

Sample Dilution factor = 5

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 Diluent 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 completely flowed through replace the syringe with a new one, elute with 1 mL of the Eluent Solution at a rate of 1 drop per second. Collect the eluate in a glass test tube. Repeat this 2 more times.
6. After elution, dry the eluate with a nitrogen evaporator at 60°C, store the residue at 4°C. Before using the residue, redissolve with 0.5 mL of Resuspension Solution and derivatize in accordance to national standard (GB5009.240-2016) methods and test; the ratio of the derivatization solution and the re-dissolution solution during derivatization is 1:1.

** The extraction procedure for this product also applies to GB5009.240—2016 "Determination of Fumonisin in Food", but washing and elution should be performed as per manufacturers' instruction manuals.*

** The product is also suited for the Food Safety Risk Monitoring Program.*

INTERPRETATION OF RESULTS

Fumonisin Content = Detected Concentration x Dilution Factor/2