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MaxSignal® IAC for DON

Vomitoxin Immunoaffinity Column

Catalog #FOOD-1508-01

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 B1, Zearalenone, Fumonisin, Vomitoxin and its derivatives	25 Tests
FOOD-1502-01	MaxSignal® IAC 4-in-1 Combo for Aflatoxin B1, Zearalenone, Vomitoxin, 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-1509-01	MaxSignal® IAC for Total DON	25 Tests

GENERAL INFORMATION

Purpose

The immunoaffinity column can selectively adsorb Deoxynivalenol (vomitoxin, DON) from the sample solution, thereby purifying the sample. The purified sample solution can be directly used for HPLC/LC-MS/MS analysis after concentrating with nitrogen gas and resuspended.

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

Deoxynivalenol also known as Vomitoxin, belongs to the trichothecene family and is produced by some Fusarium species. Vomitoxins are mostly distributed in grain seeds such as wheat, barley, and corn, and its content is usually in the range of mg/kg. Due to their high cytotoxicity and immunosuppressive properties, they pose a health threat to both humans and animals.

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 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 an deoxynivalenol 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: 2000 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.
- 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.
- WARNING: Vomitoxin is toxic and carcinogenic; protective equipment such as gloves and masks should always be used during handling.

REAGENT PREPARATION

1. **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.
2. **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.
3. **Preparation of 0.1% Tween-water**
   Take 1 ml of Tween-20, add distilled/deionized water to top up the volume to 1 L.

SAMPLE PREPARATION

Grains and Feed

Method 1 - Samples that do not absorb much water, such as corn, wheat, flour, compound feed, concentrates, supplements, etc.

1. Weigh out 25 g ± 0.01 g of sample (solid samples should be ground and passed through a 1-mm sieving screen) and place into a container; add 5 g of polyethylene glycol and 100 mL of distilled/deionized water.
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. Repeat Step 3.
5. Use 2 mL of the filtrate with the immuoaffinity column for purification.

Dilution Factor = 2

Method 2 - Samples that are strong absorbents, such as bran, soybean hull, corn germ meal, rice bran, etc.

1. Weigh out 10 g ± 0.01 g of sample (solid samples should be ground and passed through a 1-mm sieving screen), add 2 g of polyethylene glycol and 100 ml of water;
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. Repeat Step 3.
5. Use 5 mL of the sample with the immuoaffinity column for purification.

Dilution Factor = 2

Alcohols

1. Weigh out 20 g of alcohol sample (accurate to 0.1 g), add 1 g of polyethylene glycol, add distilled/deionizedwater to a final volume of 25.0 mL, mix well, and place in an ultrasonic/vortex mixer or shaker to shake for 20 minutes. Filter with glass fiber filter paper until the filtrate is clear (or centrifuge at 6000 RPM for 10 minutes), and collect the filtrate in a clean container.
2. Use 2 mL of the sample with immuoaffinity column for purification.

Dilution Factor = 0.625

Soy sauce, vinegar, sauces and soy products

1. Weigh out 25 g of sample (accurate to 0.1 g), add 5 g of polyethylene glycol, add distilled/deionized water to a final volume of 100 mL, mix well, and place in an ultrasonic/vortex mixer or shaker to shake for 20 minutes. Filter through glass fiber filter paper until the filtrate is clear (or centrifuge at 6000 RPM for 10 minutes), and collect the filtrate in a clean container.
2. Use 2 mL of the sample with the immuoaffinity column for purification.

Dilution Factor = 2

Infant formula rice flour

1. Weigh out 25 g ± 0.01 g of sample (solid samples should be ground and passed through a 1-mm sieving screen) and place into a container. Add 100 mL of Extraction Solution.
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. Transfer 10 mL of filtrate to a new tube and add 70 mL of Diluent Solution, mix well.
5. Filter with microfiber filter paper. Collect the filtrate.
6. Use 16 mL of filtrate with the immunoaffinity column for purification.

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 completely flowed through, for grains and feed samples, add 10 mL of distilled/deionized water to wash; repeat this step one more time. For the rest of the samples, first wash with 10 ml of 0.1% Tween-water solution, and the wash with 10 ml of water at flow rates of 2 to 3 drops/second;
5. After the liquid has flowed through, add 1 mL of methanol at a flow rate of 1 drop per second, collect the eluate. Concentrate the eluate at 50-60°C with nitrogen gas flow and redissolve the residue with mobile phase.
6. Filter the resuspended solution through a 0.22 µm micropore filter and then transfer into a sample bottle to be used with HPLC, LC-MS/MS, or other analytical device. for analysis.

Also applies to GB 5009.111-2016 and GB/T30956-2014.

INTERPRETATION OF RESULTS

Deoxynivalenol Concentration = Detected Concentration x Dilution Factor

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