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For the Better

MaxSignal© IAC for T-2/HT-2 Toxin

Catalog #FOOD-1512-01

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

GENERAL INFORMATION

Purpose

The immunoaffinity column can selectively adsorb T-2 and HT-2 toxins from the sample solution, thereby purifying the sample. The purified sample solution can be used for LC-MS/MS analysis after concentrated 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

T-2 toxin (T-2) is a mycotoxin produced by a variety of *Fusaria*.

It mainly pollutes food crops such as wheat, barley, and corn, and their products, and is relatively harmful to human health and cattle industry. HT-2 toxin is the main metabolite of T-2 toxin. These two toxins are strong inhibitors of protein synthesis in the body and mainly affect the functions of blood, liver, kidney, pancreatic muscle and lymphocytes. The general clinical symptoms after poisoning are anorexia, vomiting, diarrhea, growth arrest, as well as reproduction and neurological dysfunction, etc.

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 T-2/HT-2 toxin 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 Certificate of Analysis.

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 total capacity of the T-2/HT-2 column is 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.
- WARNING: T-2/HT-2 toxin is cytotoxic; 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 Methanol-water**
   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 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 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 out 25 g ± 0.01 g of sample in a bottle. Add 5 g of sodium chloride (NaCl) and 125 mL of Extraction Solution 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. Combine 10 mL of the filtrate with add 40 mL of Diluent, then filter with microfiber filter paper, and collect the filtrate.

4. Use 25 mL of the sample with immuoaffinity column for purification.

Dilution factor = 1

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 add 10 mL of distilled/deionized water a t a rate of 2-3 drops per second. Repeat this step one more time.
5. After the liquid has completely flowed through add 1 mL of 2% acetic acid-methanol solution, collect the eluent with a sample bottle at a flow rate of 1drop per second, concentrate by passing a slow stream of nitrogen at 50°C to evaporate any residual solvent.
6. LC-MS/MS testing can be conducted, or HPLC testing can be conducted after derivatization in accordance with the GB 5009.118—2016.GB/T 28718-2012PLC standards.

INTERPRETATION OF RESULTS

T-2/HT-2 Toxin Concentration = Detected Concentration of T-2/HT-2 Toxin x Dilution Factor

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