YILI BIOTECH

IAC for Deoxynivalenol

Instruction Manual

1. Purpose:

The immunoaffinity column can selectively adsorb Deoxynivalenol (Vomitoxin, DON) from the sample solution, thereby specifically purify the sample. The purified sample solution can then 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/LC-MS/MS to achieve rapid testing, and to increase signal-to-noise ratio and improve the accuracy of the detection method.

2. 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.

3. Principle:

The basis of the measurement is the antigen-antibody reaction. Antibodies are connected to the column and the Vomitoxin in the sample is extracted, filtered, and diluted, and then passed slowly through the Vomitoxin immunoaffinity column. The toxins bind to the antibodies in the column and the immunoaffinity column is then washed to remove other unrelated substances that have not been bound. Vomitoxin is then eluted with methanol and injected into an analytical instrument for detection.

4. Components of the kit:

Each kit contains Vomitoxin immunoaffinity columns of various specifications and 1 instruction manual.

5. Necessary items not provided in the box:

5.1 Equipment

- HPLC/LC-MS/MS
- Centrifuge capable of at least 3,000-4,000 RPM
- Nitrogen gas evaporator apparatus
- Nitrogen gas tank and pressure regulator
- Air-pressure controller bracket
- Air pump
- Balance with 0.01g readability
- High-speed homogenizer (maximum speed> 10,000 RPM) or shaker
- Grinder
- Sieving screen:1-mm
- Graduated cylinder: 100 mL/10 mL
- Funnel: 50 mL
- Syringe: 10 mL/20 mL
- Pipette: 1 mL and pipette tips
- Homogenization flask (or 250-mL conical flask with pestle)
- Vials and tubes
- Rapid qualitative filter paper
- Microfiber filter paper (e.g. Whatman 934-AH)
- Column holder and syringe connector plug (for use with immunoaffinity columns)

5.2 Reagents

- Methanol (CH3OH): Chromatographic grade
- Acetonitrile (CH3CN): Analytical Grade
- Sodium chloride (NaCl): Analytical Grade
- Potassium dihydrogen phosphate (KH2PO4): Analytical Grade
- Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$): Analytical Grade
- TWEEN-20 ($C_{58}H_{114}O_{26}$): Analytical Grade

Add：F4, Building D9, 1 Jinhui Road, National High-Tech Development Zone, Qingdao, China
Tel: +86-532-5826 8660 Zip: 266111 Website：www.yili-bio.com

- Polyethylene glycol (8000Da, HO(CH2CH2O)nH): Analytical Grade, to improve filtration speed, if the supernatant is obtained by centrifugation , this reagent can be omitted.
- Sodium hydroxide (NaOH): Analytical Grade
- Hydrochloric acid (HCl): Analytical Grade
- Distilled/deionized water

6. Note:

- Allow the immunoaffinity column to return to room temperature (22 to 25°C) before use.
- The affinity column should be stored at 2 to 8°C, do not freeze.
- Dot use any expired immunoaffinity column.
- The sample volume can be increased or decreased appropriately as required, and the volume of the extraction solution should be adjusted accordingly.
- Column capacity: 200 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.
- The pH of the loading solution of the affinity column should be within the range of 6 to 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.
- WARNING: Deoxynivalenol is 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 LCMS/MS is clean and the tubing is primed appropriately for each run.
- Follow appropriate instrument precautions if using HPLC.

7. Reagent preparation：

7.1 Diluent Solution: PBS:

- Weigh out 8 g of NaCl, 0.2 g of KCl, 0.2 g of KH_2PO4 and 1.16 g of $Na_2HPO_4 \cdot 12H2O$ into a large, graduated bottle. Dissolve with 800 mL of distilled/deionized water, then bring to a final volume of 1 L with distilled/deionized water. Mix well.

7.2 Extraction Solution (80% v/v acetonitrile-water solution):

- Combine 800 mL of acetonitrile and 200 mL of distilled/deionized water. Bring to 1 L final volume with distilled/deionized water. Mix well.

7.3 0.1%Tween-water solution:

- Combine 1 mL of Tween-20 with distilled/deionized water to a final volume of 1000 mL. Mix well.

8. Sample Preparation:

Grains and Feed

Method 1: Suitable for samples that do not absorb much water, such as corn, wheat, flour, compound feed, concentrates, supplements, etc

- 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 5g of polyethylene glycol and 100 mL of distilled/deionized water.
- 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.
- Filter with rapid qualitative filter paper and collect the filtrate.
- Filter with microfiber filter paper and collect the filtrate as sample solution.
- Use 2 mL of the sample solution with the immunoaffinity column for purification.

Dilution Factor = 2

Method 2: Suitable for samples that are absorb much water, such as bran, soybean husk, corn germ meal, rice bran, etc.

- Weigh out 10 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 2g of polyethylene glycol and 100 mL of distilled/deionized water.
- 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.

- Filter with rapid qualitative filter paper and collect the filtrate.
- Filter with microfiber filter paper and collect the filtrate as sample solution.
- Use 5 mL of the sample solution with the immunoaffinity column for purification.

Dilution Factor = 2

Alcohols

- Weigh out 20g ± 0.01 g of sample and place into a container; add 1g of polyethylene glycol , add distilled/deionized water 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.
- Use 2 mL of the sample with immunoaffinity column for purification.

Dilution Factor = 0.625

Soy sauce, vinegar, sauce, and sauce products

- Weigh out 25g ± 0.01 g of sample and place into a container; add 5g 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 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.
- Use 2 mL solution of the sample with immunoaffinity column for purification.

Dilution Factor = 2

Infant formula rice flour

- 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 (80% v/v acetonitrile-water solution).
- 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.
- Filter with rapid qualitative filter paper, and collect the filtrate.
- Transfer 10 mL of filtrate to a new tube and add 70 mL of Diluent Solution, mix well.
- Filter with microfiber filter paper. Collect the filtrate.
- Use 16 mL of filtrate with the immunoaffinity column for purification.

Dilution Factor = 2

9. Operating procedure:

- 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.
- Transfer the appropriate amount of the solution processed in Sample Preparation to fill the syringe.
- Remove the cap under the affinity column. Adjust the air-pressure to have a flow rate of 1–2 drops per second.
- 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 -3 drops per second.
- 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.
- Filter the resuspended solution through a 0.22 μm micropore filter and then transfer into a vial to be used with HPLC, LC-MS/MS, or other analytical device.

10. Interpretation of results:

Deoxynivalenol Concentration = Detected Concentration × Dilution Factor

11. Storage conditions and period of validity

Storage Conditions: 2 to 8°C

Expiry Date: this product is valid for a period of 18 months.

Add：F4, Building D9, 1 Jinhui Road, National High-Tech Development Zone, Qingdao, China
Tel: +86-532-5826 8660 Zip: 266111 Website：www.yili-bio.com