YILI BIOTECH

IAC for Aflatoxin Total/ Ochratoxin A/ Zearalenone 3 in 1

Instruction Manual

1. Purpose:

The immunoaffinity column can selectively adsorb aflatoxins Total (B1, B2, G1, G2), Ochratoxin A, Zearalenone from the sample solution, thereby purifying the sample. The purified sample solution can then be directly used for HPLC or LC-MS/MS analysis.

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

2. Overview:

Aflatoxins are toxic metabolites of a class of fungi (such as Aspergillus flavus and Aspergillus parasiticus), which are highly carcinogenic. Zearalenone (ZEN), also known as F-2 toxin, mainly contaminates crops such as corn, wheat, and cereals. It has a strong estrogen effect and can cause hyperestrogenism and severe reproductive tract symptoms and infertility. It also has immunotoxicity and genotoxicity. Ochratoxin A is produced by a variety of Aspergillus and Penicillium growing on crops such as grains, peanuts, and beans. Ochratoxin A mainly attacks the liver and kidneys of animals, causing liver damage and also has teratogenic effects. These three toxins often contaminate crops and their processed products at the same time. The use of a three-in-one immunoaffinity column can purify these three toxins at the same time, greatly saving pre-treatment time.

3. Principle:

The basis of the measurement is the antigen-antibody reaction. Antibodies are connected to the column and the aflatoxin in the sample is extracted, filtered, and diluted, and then passed slowly through the 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. Toxins are then eluted with methanol and injected into an analytical instrument for detection.

4. Components of the kit:

Each kit contains Aflatoxin total/Ochratoxin A/ Zearalenone 3 in 1 immunoaffinity columns of various specifications and 1 instruction manual.

5. Necessary items not provided in the box:

5.1 Equipment

- HPLC or LC-MS/MS
- Derivatization device: such as optical derivatization device, photochemical derivatization device, iodine derivatization device
- 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:1mm
- Graduated cylinder: 100 mL/10 mL
- Funnel: 50 mL
- Syringe: 10 mL/20 mL
- Pipette: 1 mL and pipette tips
- Homogenization flask (or 250mL conical flask with pestle)
- Vials and tubes
- Rapid qualitative filter paper
- Microfiber filter paper (e.g. Whatman 934AH)

- Column holder and syringe connector plug (for use with immunoaffinity columns)

5.2 Reagents

- Methanol (CH3OH): chromatographic grade
- Acetonitrile (CH3CN): analytical grade
- Potassium chloride (KCl): analytical grade
- Sodium chloride (NaCl): analytical grade
- Potassium dihydrogen phosphate (KH2PO4): analytical grade
- Sodium hydrogen phosphate dodecahydrate (Na2HPO4·12H2O): analytical grade
- Acetic acid (CH3COOH): chromatographic grade
- Tween-20 (C58H114O26): analytical grade
- Hydrochloric acid (HCl): analytical grade
- Sodium hydroxide (NaOH): analytical grade
- Water (H2O): distilled water or 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: Aflatoxins(300ng), Ochratoxin A(100ng), Zearalenone(2000ng) 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: Aflatoxin 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 Extraction Solution 1(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.

7.2 Diluent: 0.01M pH7.3 PBS:

- Weigh 8g NaCl, 0.2g KCl, 0.2g KH2PO4 and 1.16g Na2HPO4·12H2O, add 800mL distilled water or deionized water to dissolve and adjust the volume to 1L.

7.3 0.1% Tween-water solution:

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

7.4 Eluent: 2% acetic acid-methanol:

- Pipette 2 mL of acetic acid and add 98 mL of methanol and mix well.

8. Sample Preparation:

- 25g±0.01g sample (solid sample needs to be crushed and passed through a 2mm sample sieve) is extracted with 125mL of extracting solution 80% acetonitrile-water solution (acetonitrile: water = 80:20);
- High-speed homogenization (≥10,000r/min) for 1min, or vigorous shaking on a shaker (200r/min～
300r/min) for 20min;
- Filter with fast qualitative filter paper or centrifuge at 4000rpm for 5min;
- Take 10mL of filtrate (or supernatant after centrifugation) and add 70mL of diluent to dilute and mix;
- Filter with microfiber filter paper until clear;
- Take 20mL of filtrate (equivalent to 0.5g sample) for sample loading test.

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 airpressure 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 is drained, load 2mL of eluent (acetic acid: methanol = 2:98). When the liquid fills the column filler, plug the plug below the affinity column and let it stand for 3 minutes. Then elute at a flow rate of 1 drop/second and collect the eluent;.
- Blow it to near dryness with nitrogen at 50℃ and reconstitute it with 1mL of mobile phase;
- Filter the reconstituted solution with a 0.22μm microporous filter and transfer it to a sample bottle for LC-MS (LC-MS/MS) analysis.
- Before each loading, the previous liquid must be completely drained.

10. Interpretation of results:

Aflatoxins Concentration = Detected Concentration × Dilution Factor

Ochratoxin A Concentration = Detected Concentration × Dilution Factor

Zearalenone 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.