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YILI BIOTECH

IAC for Ochratoxin A

Instruction Manual

1. Purpose:

The immunoaffinity column can selectively adsorb Ochratoxin A 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 HPLC analysis or for LC-MS/MS analysis after being concentrated with nitrogen gas and resuspended.

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

2. Overview:

Ochratoxin A (OTA) is a toxic metabolite produced by some species of Aspergillus and Penicillium, it is a mycotoxin with high kidney and liver toxicities, and has teratogenic, mutagenic, and carcinogenic effects.

3. Principle:

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

4. Components of the kit:

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

5. Necessary items not provided in the box:

5.1 Equipment

- HPLC
- 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 r/ min) or shaker
- Grinder
- Sieving screen:2-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 (CH_3OH): Analytical grade for extraction/Chromatography grade for elution
- Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$): Analytical Grade
- Acetic acid (CH_3COOH): Chromatography Grade
- Potassium dihydrogen phosphate (KH2PO4): Analytical Grade
- Sodium chloride (NaCl): Analytical Grade
- TWEEN-20 ($C_{58}H_{114}O_{26}$): Analytical Grade
- Hydrochloric acid (HCl): Analytical Grade
- Sodium hydroxide (NaOH): Analytical Grade
- Potassium chloride (KCl): Analytical Grade
- Sodium Bicarbonate ($NaHCO_3$):Analytical Grade
- Distilled or deionized water

6. Note:

- Allow the immunoaffinity column to return to room temperature(22 to 25°C) before use.

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- The immunoaffinity 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: 100 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 immunoaffinity 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 LC-MS/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 (For the extraction of grain samples and soy sauce and vinegar samples):

- Combine 800 mL of methanol to bring to 1 L final volume with distilled/deionized water. Mix well.

7.2 Extraction Solution 2 ((For the extraction of alcoholic samples):

- Weigh out 150 g of sodium chloride and 20 g of sodium bicarbonate and dissolve in approximately 950 mL of distilled/ deionized water, mix, then bring to a final volume of 1 L with distilled/deionized water.

7.3 Extraction Solution 3 ((For the extraction of spices samples):

- Combine 400mL 1% sodium bicarbonate solution and 600mL methanol, mix well. Among them 1% sodium bicarbonate solution: Weigh out 10.0g of sodium bicarbonate and dissolve in distilled/ deionized water, then bring to a final volume of 1L with distilled/deionized water.

7.4 Extraction Solution 4 ((For the extraction of star anise):

- Combine 200mL 3% sodium bicarbonate solution and 800mL methanol, mix well. Among them 3% sodium bicarbonate solution: Weigh out 30.0g of sodium bicarbonate and dissolve in distilled/deionized water, then bring to a final volume of 1L with distilled/deionized water.

7.5 Extraction Solution 1(For the extraction of coffee):

- Combine 500mL 3% sodium bicarbonate solution and 500mL methanol, mix well. Among them 3% sodium bicarbonate solution: Weigh out 30.0g of sodium bicarbonate and dissolve it in distilled/ deionized water, then bring it to a final volume of 1L with distilled /deionized water.

7.6 Wash Solution 1 (For washing of soy sauce and vinegar samples) :

- Combine 12.50 g of sodium chloride and 2.5 g of sodium bicarbonate and dissolve in water, add 0.1 ml of Tween-20 and bring to a final volume of 1 L with distilled/deionized water.

7.7 Wash Solution 2(for washing in the purification step for alcoholic samples) :

- Combine 25 g of sodium chloride and 5 g of sodium bicarbonate and dissolve in approximately 950 mL of distilled/deionized water, mix, then bring to a final volume of 1 L with distilled/deionized, and top up the volume with water to 1 L.

7.8 Wash Solution 3 (for washing in the purification step for spice samples) :

- 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 with distilled/ deionized water,and add 1 mL of Tween-20,mix well.

7.9 Diluent or Wash Solution 4 (for extraction, dilution, and washing in the purification step for coffee samples):

- Weigh out 8.0 g of NaCl, 0.2 g of KCl, 0.2 g of KH_2PO_4, and 1.2 g of Na_2HPO_4 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, add 1 mL of Tween-20, mix, and well.

7.10 Eluent (for elution of all samples after they pass through the column)

- Combine 2 mL of acetic acid and 98 mL of methanol. Mix well.

8. Sample Preparation:

Method 1: Grains and Chili

- Weigh out 20 g ± 0.01 g of sample in a bottle. Add 5 g of sodium chloride (NaCl) and 100 mL of **Extraction Solution 1**. Solid samples should be homogenized to pass through a 2-mm sieve before use.
- Homogenize at high speed (≥10,000 RPM) for 1 min, or shake vigorously on a shaker (200 RPM to 300 RPM) for 20 min, filter with a rapid qualitative filter paper, and collect the filtrate;
- Combine 10 mL of the eluent with 40 mL of distilled/deionized water to dilute, mix well.
- Filter with microfiber filter paper, and collect the filtrate as sample solution.
- Use 25 mL of the sample solution with the immunoaffinity column for purification.

Dilution factor: 1

Method 2: Alcohols

- Take 20 g ± 0.01 g of a degassed alcohol sample (for alcohol samples containing carbon dioxide, stir or degas using ultrasound before use) or an alcohol sample without carbon dioxide, add **Extraction Solution 2** and bring to a final volume of 25 mL.
- Homogenize at high speed (≥10,000 RPM) for 1 min, or shake vigorously on a shaker (200 RPM to 300 RPM) for 20 min, then filter with microfiber filter paper, and collect the filtrate as the sample solution.
- Use 1 mL of the sample solution with the immunoaffinity column for purification.

Dilution factor: 1.25

Method 3: Soy sauce and vinegar samples

- Take 25 g ± 0.01 g of sample, add **Extraction Solution 1** and bring to volume of 50 mL.
- 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 a rapid qualitative filter paper, and collect the filtrate;
- Combine 10 mL of the eluent ,bring to a final volume of 50 mL with distilled/deionized water, mix well.
- Filter with microfiber filter paper, and collect the filtrate as sample solution.
- Use 25 mL of the sample solution with the immunoaffinity column for purification.

Dilution factor:0.4

Method 4: Spices samples, such as curry,cinnamon and pepper

- Weigh 20 g ± 0.01 g of sample into a bottle. Add to 100 mL of **Extraction Solution 3**. Solid samples should be homogenized to pass through a 2-mm sieve before use.
- 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 microfiber filter paper. Collect the filtrate.
- Combine 10 mL of the filtrate with 40 mL of distilled/deionized water to dilute, mix well.
- Filter with microfiber filter paper,and collect the filtrate as sample solution.
- Use 25 mL of the sample solution with the immunoaffinity column for purification.

Dilution factor: 1

Method 5: Star anise

- Weigh 20 g ± 0.01 g of sample into a bottle. Add to 100 mL of **Extraction Solution 4**. Solid samples should be homogenized to pass through a 2-mm sieve before use.
- 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 microfiber filter paper. Collect the filtrate.
- Combine 10 mL of the filtrate with 40 mL of distilled/deionized water to dilute, mix well. Centrifuge at 12,000RPM for 5min.
- Filter with microfiber filter paper, and collect the filtrate as sample solution.

Dilution factor: 1

Method 6: Coffee (including toasted coffee bean, green coffee bean, instant coffee, etc.)

- Weigh 1.0 g ± 0.01 g of sample into a bottle. Add 20 mL of **Extraction Solution 5**. Solid samples should be homogenized to pass through a 2-mm sieve before use.
- Homogenize, such as vortex, at high speed (≥10,000 RPM) for 3 minutes or shake vigorously on a shaker (200-300 RPM) for 20 minutes.
- Centrifuge at 4,000 RPM for 5min. Or filter with a rapid qualitative filter paper. Collect the supernatant or filtrate.
- Combine 5 mL of the supernatant or filtrate with 20 mL **Diluent** to dilute and mix well.
- Filter with microfiber filter paper, and collect the filtrate as a sample solution.
- Use 20 mL of the sample solution with the immunoaffinity column for purification.

Dilution factor: 5

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 (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.
- If the sample to be purified is a grain, after the liquid has completely flowed through, add 10 mL of distilled/deionized water at a flow rate of 2-3 drops per second.
- If the sample to be purified is soy sauce or vinegar, after the liquid has completely flowed through, add 10 mL of **Wash Solution 1** at a flow rate of 2-3 drops per second.
- If the sample to be purified is an alcohol, after the liquid has completely flowed through, add 10 mL of **Washing Solution 2**, then 10 mL of water at a rate of 2-3 drops per second.
- If the sample to be purified is a spice or star anise, after the liquid has completely flowed through, add 10 mL of **Washing Solution 3**, then 10 mL of water at a rate of 2-3 drops per second.
- If the sample to be purified is coffee, after the liquid has completely flowed through, add 10 mL of **Washing Solution 4**, then 10 mL of water at a rate of 2-3 drops per second.
- After the liquid has completely flowed through replace the syringe with a new one, add 2 mL of **Eluent**, use a test tube to collect the eluent at a flow rate of 1 drop per second, collect the eluent and bring to volume of 2 mL .

- Filter the eluent through a 0.22 μm micropore filter and then transfer into a vial to be used for HPLC analysis.

10. Interpretation of results:

Ochratoxin A Concentration= Detected Concentration ×Dilution Factor ×2

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.