Elaboric

The SEPurast IAC for Aflatoxins B_1,B_2,G_1 and G_2

Instruction Manual

Product No. IAC02251 / IAC02502 / IAC02253 / IAC02504

1. Introduction

This immunoaffinity column is designed for the specific extraction and enrichment of aflatoxins B_1, B_2, G_1, and G_2. It efficiently captures the target toxins from complex sample matrices, providing highly purified extracts to ensure accurate and reliable results for subsequent HPLC or LC-MS/MS analysis.

Aflatoxins are a group of highly toxic secondary metabolites, with B_1, B_2, G_1, and G_2 being the primary types, among which Aflatoxin B_1 exhibits the highest toxicity. Primarily produced by the fungal species such as *Aspergillus flavus* and *Aspergillus parasiticus*, they frequently contaminate susceptible agricultural commodities such as corn, peanuts, legumes, and tree nuts. Exposure to these toxins can lead to hepatotoxicity, immunosuppression, and hepatocellular carcinoma. Notably, Aflatoxin B_1 is recognized as one of the most potent naturally occurring carcinogens known.

The column operates on the principle of specific antigen-antibody binding. High-affinity antibodies against aflatoxins B_1, B_2, G_1 and G_2 are covalently immobilized on the gel medium. As the sample extract passes through the column, the antibodies selectively capture the aflatoxins, forming a stable complex, while interfering matrix components are removed during the washing step. Finally, the antigen-antibody complex is disrupted using methanol, eluting the aflatoxins to yield a highly purified sample ready for subsequent HPLC or LC-MS/MS analysis.

2. Information of the Validation

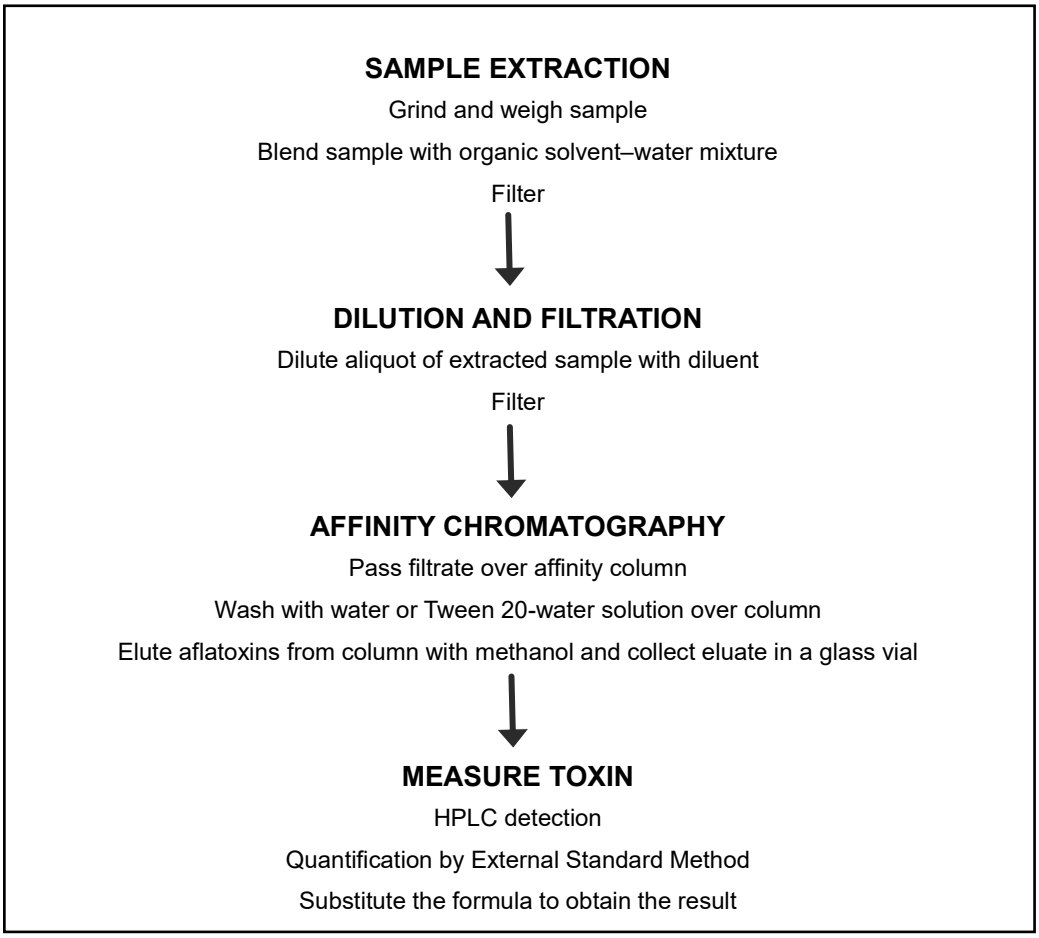
AOAC Performance Tested Methods Certificate #number

This product has successfully obtained AOAC Official Certification. According to AOAC *Performance Tested Methods*^{SM} (*PTM*) study, the method described in the instruction manual has been validated for the analysis of corn and chili powder.

3. General information of the IAC

IAC Name	Total Aflatoxins (B_1,B_2,G_1,G_2) IAC
Specification	1 mL / 3 mL, 25 or 50 tests per box
Column capacity	300ng
Instruction manual	One per box
COA	One per box
Type of Service	Quantitative
Storage conditions and period of validity	18 months at 2–8 ℃
Approved Commodities	Cereal, Nuts, Spices, Feed, Condiment
Detection Method	HPLC or LC-MS/MS

4. IAC Method Overview



5. Necessary items not provided in the box

5.1 Equipment

Name	Specifications
Nitrogen Evaporator	/
Balance	0.01 g readability
High-speed homogenizer	Speed ≥ 10,000 rpm
High-speed herb grinder	e.g., CGOLDENWALL CNA 923D
Centrifuge	Speed ≥ 4,000 rpm
Sieving screen	1 mm
Graduated cylinder	1000 mL
Syringe	10 mL / 20 mL
Pipette	1 mL, 10 mL and pipette tips
Conical flask	250 mL
Glass vials	3 mL
Rapid qualitative filter paper	e.g., Whatman 1001-090
Glass microfiber filter paper	e.g., Whatman 934AH
Column holder	with gas pressure controller

5.2 Reagents

Name	Grade
Methanol (CH_3OH)	Analytical grade for extraction; HPLC grade for elution
Acetonitrile (CH_3CN)	Analytical grade
Sodium chloride (NaCl)	Analytical grade
Potassium chloride (KCl)	Analytical grade
Potassium dihydrogen phosphate (KH_2PO_4)	Analytical grade
Disodium hydrogen phosphate (Na_2HPO_4)	Analytical grade
Tween-20 ($C_{58}H_{114}O_{26}$)	Analytical grade

6. Precautions

- Allow the immunoaffinity column to equilibrate to room temperature (20–25 °C) before use. Do not freeze.
- Sample size may be adjusted, but keep the extraction ratio constant.
- Loading solution pH must be 6–8; adjust with dilute HCl or NaOH if needed.
- The analyte solvent should be aligned with the mobile phase to mitigate solvent effects during instrumental analysis.
- Mycotoxins are highly hazardous substances. Appropriate personal protective equipment (PPE), including gloves, safety glasses and lab coats should be worn throughout the analysis. All containers and tools that come into contact with toxin solutions should be completely immersed in a (5% v/v) sodium hypochlorite solution overnight.

7. Reagent Preparation

70% methanol-water solution

This solution can be kept for 1 month if stored at room temperature.

- 700 mL Methanol (CH_3OH)
- 300 mL Water

80% methanol-water solution

This solution can be kept for 1 month if stored at room temperature.

- 800 mL Methanol (CH_3OH)
- 200 mL Water

80% acetonitrile-water solution

This solution can be kept for 1 month if stored at room temperature.

- 800 mL Acetonitrile (CH_3CN)
- 200 mL Water

Tween 20-water solution

This solution can be kept for 5 days if stored at room temperature.

Solution desired	Tween-20 (mL)	Water	Total volume (mL)
1% Tween 20-water	10	990	1000
10% Tween 20-water	100	900	1000
15% Tween 20-water	150	850	1000

PBS buffer

This buffer can be kept for 5 days if stored at room temperature.

1. Dissolve the following into approximately 900 mL distilled water
   - 8.0 g Sodium chloride (NaCl)
   - 0.2 g Potassium chloride (KCl)
   - 1.2 g Disodium hydrogen phosphate (Na_2HPO_4)
   - 0.2 g Potassium dihydrogen phosphate (KH_2PO_4)
2. Adjust pH to 7.0 with concentrated Hydrochloric Acid (HCl)
3. Quantitatively transfer the solution to a 1 L volumetric flask, dilute to the mark with distilled water, and mix thoroughly.

CAUTION: Keep container tightly capped when not in use. Solution volume may be increased or decreased as needed provided the proportion of reagents is kept consistent.

8. Sample Preparation

Cereal, Nuts (corn, wheat, hazelnut, pine nuts, peanut and peanut butter, etc)

Dilution factor: 1

1) Weigh 25.00 ± 0.01g of ground sample with 5.0 g NaCl into a 250 mL conical flask.
2) Add 125 mL of 70% methanol-water solution (v/v).
3) Blend at high speed (≥ 10,000rpm) for 2 minutes.
4) Filter extract with rapid qualitative filter paper.
5) Dilute 10 mL of filtrate with 20 mL distilled water. Mix well.
   (**Note**: for complex matrix samples[1], it is recommended to use 1% Tween 20-water solution.)
6) Filter dilute extract through glass microfiber filter paper and collect the filtrate.
7) Take 15 mL (equivalent to 1.0 g of sample) of filtrate for purification by immunoaffinity column.

1 Criteria for Complex Matrices: If any of the following situations occur, a sample is generally considered a “complex matrix”. **a.** After sample processing, significant emulsification occurred when diluted with distilled water; **b.** After sample processing and dilution, the diluted extract remains significantly turbid even after passing through a glass microfiber filter; **c.** The recovery rate for spiked or quality control samples falls below the acceptable lower limit specified by the method.

Spices (chili powder, tragacanth gum, tamarind and mango powder, etc)

Dilution factor: 2

1) Weigh 25.00 ± 0.01g of ground sample with 5.0 g NaCl into a 250 mL conical flask.
2) Add 100 mL of 80% methanol-water solution (v/v).
3) Blend at high speed (≥ 10,000rpm) for 2 minutes.
4) Filter extract with rapid qualitative filter paper.
5) Measure 40mL Tween 20-water solution using a graduated cylinder. Dilute 10 mL of filtrate with the 10% Tween 20-water solution. Mix well.
6) Filter dilute extract through glass microfiber filter paper and collect the filtrate.
7) Take 10 mL (equivalent to 0.5 g of sample) of filtrate for purification by immunoaffinity column.

Complex matrix spices (pomegranate seeds, star anise and turmeric, etc)

Dilution factor: 2

1) Weigh 25.00 ± 0.01g of ground sample into a 250 mL conical flask.

2) Add 100 mL of 80% acetonitrile-water solution (v/v).
3) Blend at high speed (≥ 10,000rpm) for 2 minutes.
4) Filter extract with rapid qualitative filter paper.
8) Measure 40mL Tween 20-water solution using a graduated cylinder. Dilute 10 mL of filtrate with the 15% Tween 20-water solution. Mix well.
5) Filter dilute extract through glass microfiber filter paper and collect the filtrate.
6) Take 10 mL (equivalent to 0.5 g of sample) of filtrate for purification by immunoaffinity column.

Feed (DDGS, soybean meal, compound feed, cloves, etc)

Dilution factor: 2

1) Weigh 25.00 ± 0.01g of ground sample into a 250 mL conical flask.
2) Add 125 mL of 80% acetonitrile-water solution (v/v).
3) Blend at high speed (≥ 10,000rpm) for 2 minutes.
4) Filter extract with rapid qualitative filter paper.
5) Dilute 10 mL of filtrate with 70 mL of 1% Tween 20-water solution. Mix well.
6) Filter dilute extract through glass microfiber filter paper and collect the filtrate.
7) Take 20 mL (equivalent to 0.5 g of sample) of filtrate for purification by immunoaffinity column.

Condiment (soy sauce and vinegar, etc)

Dilution factor: 2.5

1) Weigh 20.00 ± 0.01g of sample into a 250 mL conical flask.
2) Add methanol or acetonitrile to achieve a final volume of 100mL.
3) Blend at high speed (≥ 10,000rpm) for 2 minutes.
4) Filter extract with rapid qualitative filter paper.
5) Dilute 8 mL of filtrate with 92 mL of PBS buffer. Mix well.
6) Filter dilute extract through glass microfiber filter paper and collect the filtrate.
7) Take 25 mL (equivalent to 0.4 g of sample) of filtrate for purification by immunoaffinity column.

CAUTION: If you encounter unsatisfactory test results for complex matrix samples, please contact Elaboric for solutions.

9. Operating Procedure

1) Take out the column and place it on the column rack. Connect an empty syringe barrel securely through the cap on the top of the column. If a gas pressure controller is utilized, ensure it is properly connected.
2) Transfer the prepared sample filtrate into the syringe barrel.
3) Remove the cap under the affinity column. Apply the air pressure to adjust the flow rate to 1-2 drops per second.
4) Once all the sample filtrate has flowed through, wash the column with 20 mL distilled water at a flow rate of 2-3 drops per second. Pass air through the column for several seconds to expel any residual liquid. Carefully dry the interior walls of the column using tissue paper, strictly avoiding any contact with the column bed.

   (**Note**: If the sample solution (such as chili powder) causes the column to darken in color, wash the column with 10 mL of 1% Tween 20-water buffer and 10 mL of water in sequence.)
5) Add 1 mL of methanol, elute the aflatoxins at a controlled flow rate of 1 drop per second, applying gentle pressure to collect the entire eluate into a clean glass vial.

   (**Note**: the eluent should be collected exclusively in a clean glass vial. The use of plastic containers may lead to analyte loss due to the adsorption of aflatoxins onto plastic surfaces.)
6) Following the methanol elution, pass 1.5 mL of distilled water through the column and collect in the same vial, yielding a final combined volume of 2.5 mL.

7) Vortex the vial thoroughly to ensure a homogeneous mixture, then transfer into an autosampler vial for HPLC or LC-MS/MS analysis.

10. Interpretation of Results

Total Aflatoxins Content = Detected Concentration × Dilution Factor × 2.5

© Elaboric

Manufacturer: Elaboric Biotechnology Co., Ltd.