Manual of Aflatoxin Total (AFT) Immunoaffinity Column

1. Application

This product is used for quantitative detection of AFT by using HPLC, HPLC-MS\MS or Specialized Rapid Tester.

It is suitable for purification of AFT in samples such as cereal and feed, in order to reduce matrix interference and thus improve analysis accuracy. The purified extracting solution can be detected by different analytical methods.

2. Product Performance

Column Capacity：≥200 ng/vial
Recovery Rate：85%~110%
Column Gel：**Agarose Gel**
Advantages：**Large loading capacity, Monoclonal antibody site-specific conjugation, Easy to elute, High recovery rate.**

3. Measuring Principle

Determination of aflatoxin total (AFT) immunoaffinity column is based on the antigen-antibody reaction. AFT monoclonal antibody is coupled to agarose gel material. After extracting, filtering and diluting AFT in sample, sample extracting solution slowly passes through the Immunoaffinity Column. AFT in sample extracting solution combines with specific monoclonal antibody, meanwhile, impurities are eluted from immunoaffinity column along with washing solution. Finally, AFT is eluted by using methanol.

4. Product Composition

20 vial/box
1* manual

5. Sample Preparation and Purification

1). Weigh 20g pulverized sample, then add 100mL 70% methanol water (V:V), and then velocity mix them in homogenizer for 2minutes or vibrate for 30minutes and extract later.
2). Centrifuge at 5000r/min for 5 minutes or filter through glass fiber filter paper. Later, take 10mL filtrate, and then add 20mL water to dilute and mix well.
3). Filter by rapid qualitative filter paper or quantitative filter paper or glass microfiber filter paper, then collect filtrate.
4). Take 15mL filtrate for passing immunoaffinity column.

Note:
1. For sample extracting solution (pH<6 or pH>8), it is necessary to adjust pH value to neutral and use **glass microfiber filter paper/quantitative filter paper.**
2. For samples that are difficult to filter because of turbidity, centrifuge samples for separation.

Reference:
National standard method: "GB 5009.22-2016 National Food Safety Standard Determination of Aflatoxin B & G in Food"

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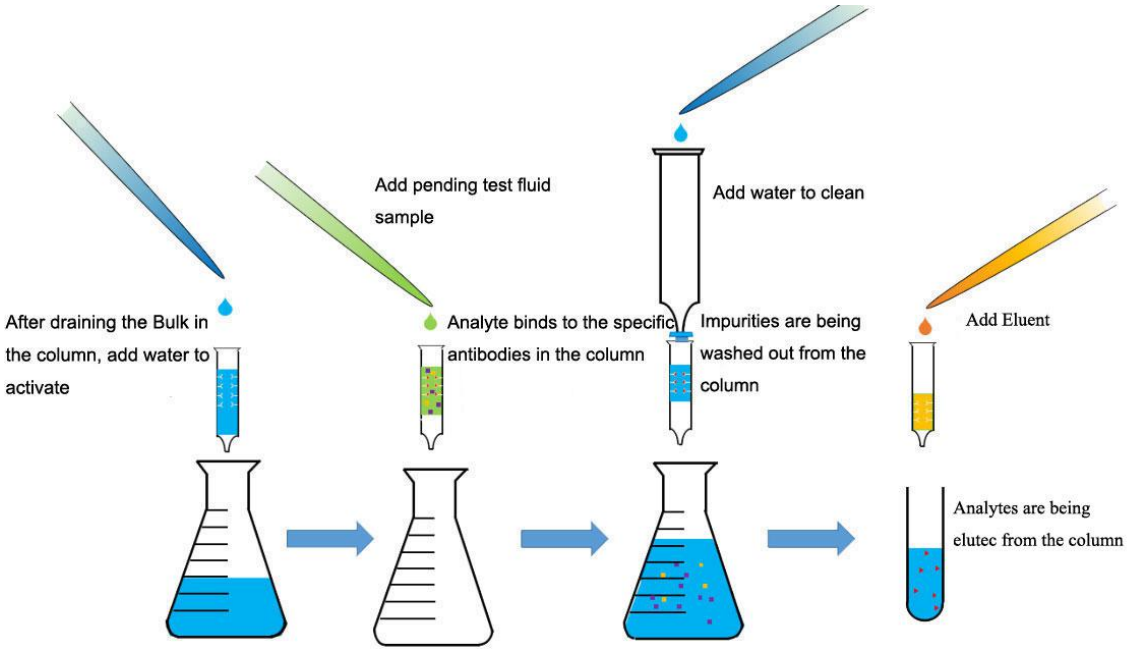
6. Immunoaffinity Column Purification

Note: Do not let column liquid drain dry before step 4.

1) Take out immunoImmunoaffinity Column microcolumn, then take off upward plug and cut it. And then plug it back into immunoaffinity column.
2) Connect the column with glass syringe on pump flow operation frame, then take off downward plug. Later, wash it once with 15mL water or phosphate buffer solution (PBS) at pH7.4 at flow rate of 1-1.5mL/min.
3) Add sample extracting solution at flow rate of 1-1.5mL/min.
4) Wash it twice with 15mL water or phosphate buffer solution (PBS) at pH 7.4 at flow rate of 1-1.5mL/min, until 2~3 mL air passes through the column to ensure that there is no residual liquid in the column.
5) Elute with 1mL methanol at flow rate of 1mL/min, and use sample bottle/glass tube to collect the eluent.
6) The collected eluent can be used for detection.



Immunoaffinity Column Operation Diagram

7. HPLC Instrument Measurement Condition

Chromatographic Column: C18, 5μm，4.6 mm×250 mm
Mobile Phase: Methanol: Water is 45:55 (V:V)
Flow Rate: 1.0mL/min
Detection Wavelength: Excitation Wavelength 360nm, Emission Wavelength 440nm
Sample Loading Quantity: 10μL
Column Temperature: 25℃

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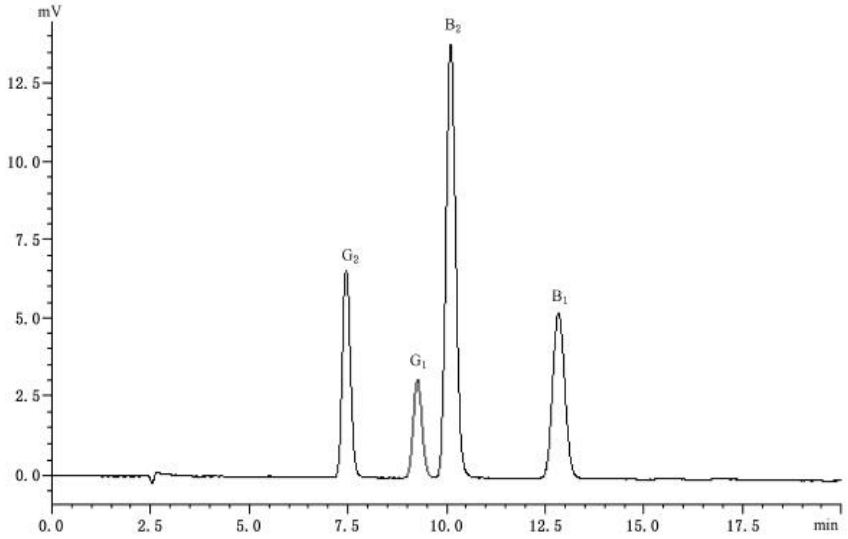

Aflatoxin Total Standard Liquid Chromatogram

8. Expiry Date

Expiry Date is 2 years.

Manufacture Date refers to information on package.

9. Note

1) AFT is harmful to human, so please wear gloves while operation. All glassware exposed to standard/sample should be soaked overnight with 5% sodium hypochlorite solution.

2) Do not use expired immunoaffinity column.

3) Immunoaffinity column should be stored at 2~8℃. Do not freeze.

4) Please put immunoaffinity column at room temperature (25℃) for half an hour at least before use.

5) If content of AFT in sample is higher than column capacity, please decrease sample loading volume accordingly.

6) Make sure that pH value of sample extracting solution that passes the column is between 6 and 8. You can adjust it with hydrochloric acid solution or sodium hydroxide solution.

7) Amount of sample weighed and volume of extracting solution can be adjusted in proportion according to actual situation. It is recommended to take 10g sample in minimum.

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