Manual of Aflatoxin M1 (AFM1) Immunoaffinity Column

1. Application

This product is used for quantitative detection of AFM1 by using HPLC, HPLC-MS\MS or Specialized Rapid Tester.

It is suitable for purification of AFM1 in samples such as milk and milk product, 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：≥100 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 M1 (AFM1) immunoaffinity column is based on the antigen-antibody reaction. AFM1 monoclonal antibody is coupled to agarose gel material. After extracting, filtering and diluting AFM1 in sample, sample extracting solution slowly passes through the Immunoaffinity Column. AFM1 in sample extracting solution combines with specific monoclonal antibody, meanwhile, impurities are eluted from immunoaffinity column along with washing solution. Finally, AFM1 is eluted by using methanol.

4. Product Composition

20 vial/box
1* manual

5. Sample Preparation and Purification

1). Liquid milk, yogurt
Weigh 4g well-mixed sample (accurate to 0.001g) and put it into 50mL centrifuge tube, then add 10mL methanol, and whirl it for 3minutes. Centrifuge at 6000r/min for 10 minutes at 4℃ or filter through glass fiber filter paper. Later, transfer appropriate amount of supernatant or filtrate into beaker, and then add 40mL water or PBS to dilute.

2). Milk powder, special dietary food
Weigh 1g sample (accurate to 0.001g) and put it into 50mL centrifuge tube, then add 4mL 50℃ hot water, and whirl it until it is well mixed. If milk powder can not be completely dissolved, put centrifuge tube into 50℃ water bath, then take it out when milk powder is totally dissolved. Cool sample solution to 20℃, then add 10mL methanol and whirl it for 3minutes. Centrifuge at 6000r/min for 10 minutes at 4℃ or filter through glass fiber filter paper. Later, transfer appropriate amount of supernatant or filtrate into beaker, and then add 40mL water or PBS to dilute.

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.

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Reference:

National standard method: "GB 5009.24-2016 National Food Safety Standard Determination of Aflatoxin M in Food"

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.

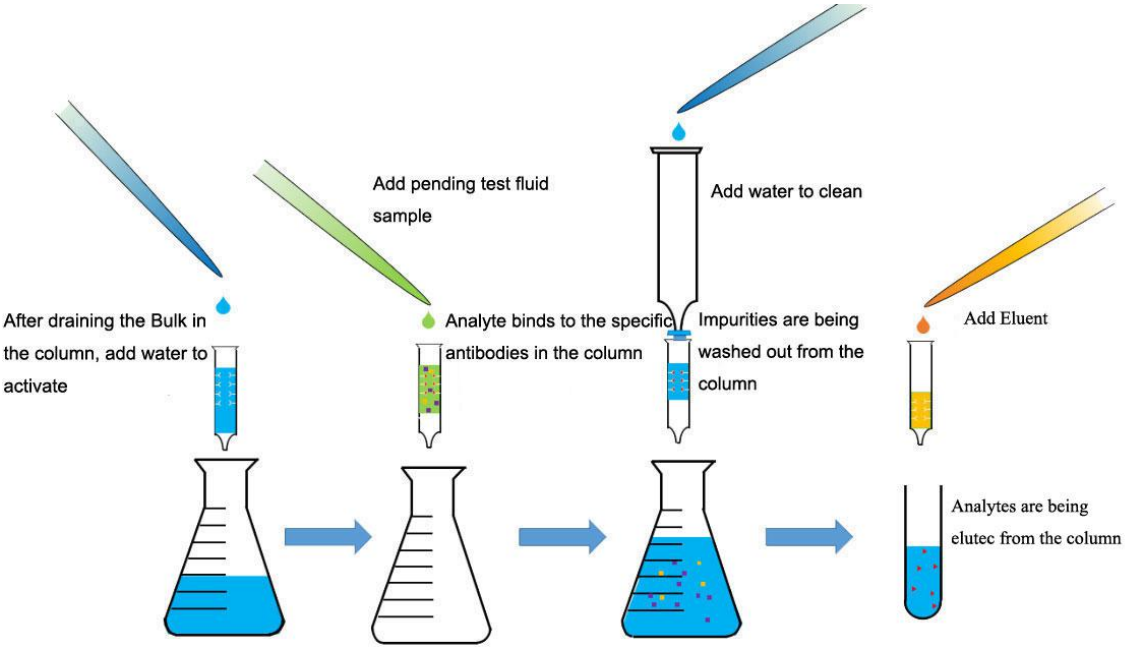
2) Connect the column with glass syringe on pump flow operation frame, then take off downward plug. Later, wash it once with 15mL water or phosphate buffer solution (PBS) at pH7.4 at flow rate of 1-1.5mL/min.

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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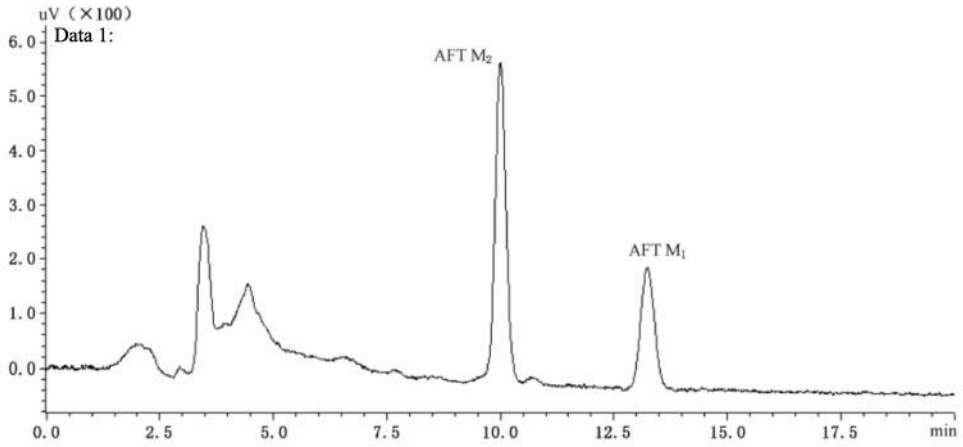

Aflatoxin M1 Standard Liquid Chromatogram

8. Expiry Date

Expiry Date is 2 years.

Manufacture Date refers to information on package.

9. Note

1) AFM1 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 AFM1 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 5g sample in minimum.

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