BIOEASY

Immunoaffinity Column for Aflatoxin M1

Order code: YRIAC3002-1C

Introduction

AFM1 belongs to a class of structurally similar compounds of aflatoxins (AFs). After mammals ingest feed or food contaminated with AFB1, a part of AFB1 accumulates in the edible parts of animals, such as liver, kidney, blood, muscle and eggs, and another part of AFB1 is converted into AFM1 and exists in the milk secreted and urine produced by animals. Its excretion and AFB1 intake were positively correlated.

AFM1 can also be directly produced by some Aspergillus flavus and Aspergillus parasiticus, but the proportion is quite low compared to other toxins (e. g. B1, B2, G1, G2).

The toxicity of AFM1 is mainly manifested in carcinogenicity and mutagenicity. The carcinogenicity of AFM1 is basically similar to that of AFB1, but its toxicity is lower than that of AFB1. However, compared with potassium cyanide and arsenic trioxide, it is still a particularly highly toxic substance and is a strong carcinogen.

Principle

Based on the specific binding of antibody and antigen, the monoclonal antibodies of AFM1 were fixed in the column to make an immunoaffinity column.

After the sample is extracted, centrifuged or filtered, and the supernatant is appropriately diluted, the AFM1 in the sample slowly passes through the immunoaffinity column and specifically binds to the antibody. Rinse the immunoaffinity column to remove other substances that are not bound and elute AFM1 with methanol. It can be used for analytical instrument detection after proper dilution.

Application

This product is suitable for the pre-processing of samples containing AFM1.

After the sample solution is purified by the immunoaffinity column, it can be directly used for qualitative and quantitative detection by HPLC, LC-MS, and other analytical instruments. It can effectively improve the signal-to-noise ratio and increase the sensitivity and accuracy of the detection method.

Solid Sample

Whole cow milk powder, whole goat milk powder, formula food for infant and young children (0-36 months): such as 1st/ 2nd/ 3rd stage goat milk powder, 1st/ 2nd/ 3rd stage cow milk powder, etc., milk powder for special dietary uses: such as pregnant and lying-in women milk powder, etc.

Liquid Sample

Liquid milk samples, such as fresh raw cows' milk, fresh raw goats' milk, pure milk, etc.

Semi-liquid sample

Cream, cheese, etc.

【Note: Please select the extraction method according to the type of sample for samples not listed in detail. If in doubt, please contact the product manager or send samples to BIOEASY for confirmation method.】

Performance Information

Column capacity: 100ng

Column recovery rate: ≥80%

Storage and Shelf Life

Storage: Store at 2-8℃(Don't be close to the inner wall of the refrigerator.) Do not freeze. Keep away from direct sunlight, moisture and heat.

Shelf Life: 12 months.

Components (25 PCs/Kit)

1. 25 PCs of 3 mL Immunoaffinity Column
2. 1 instruction manual

Materials Required but not Provided (Available from BIOEASY)

Equipment:

1. Homogenizer, high-speed pulverizer, tissue pulverizer
2. Sieve: 1mm-2mm aperture test sieve
3. Balance: 0.01 g sensibility
4. Vortex mixer
5. Ultrasonic or vortex shaker

Shenzhen Bioeasy Biotechnology Co., Ltd.

ADD: Bioeasy Building 101 (whole building), 1st Liuxian Street, Xingdong Community, Xin'an Road, Bao'an, 518101 Shenzhen, Guangdong Province, PEOPLE'S REPUBLIC OF CHINA

TEL: +86-4001111126 /+86-755-27948546 **FAX:** +86-755-27948417 **Email:** info@bioeasy.com **Web:** www.bioeasy.com

6. High-speed homogenizer: 6500 r/min-24000r/min
7. Centrifuge: speed⩾6000 r/min
8. Solid Phase Extraction device (with a vacuum pump)
9. Termovap Sample Concentrator
10. Analytical instruments such as Liquid Chromatography and Liquid Chromatograph-Mass Spectrometer, etc.
11. 50mL graduated cylinder
12. Single channel pipette: 10 μL-100 μL, 100 μL-1000 μL, 1000 μL-5000 μL
13. pH meter (or pH test strip)

Consumables:

1. Centrifuge tube (4mL, 50mL)
2. Syringe or loading tube (30mL)
3. Glass microfiber filters: required fast, high load, particle retained in liquid is 1.6 μm
4. 1mL Disposable syringe
5. Disposable millipore filter head with 0.22 μm millipore filter membrane (Test the selected filter membrane with the standard solution to confirm no adsorption phenomenon before it can be use).

Reagents

(Unless otherwise specified, all reagents used are of analytical grade, and the water is pure water.)

1. Methanol: chromatographic purity
2. Acetonitrile: chromatographic purity
3. Sodium chloride
4. Phosphate Buffer Solution (PBS) with pH 7.4: Weigh 8.00g sodium chloride, 1.20g disodium hydrogen phosphate, 0.20g potassium dihydrogen phosphate, and 0.20g potassium chloride, dissolve in water and dilute to 1000mL.
5. Acetonitrile-saturated n-hexane solution: Add 200 mL acetonitrile to 800 mL n-hexane, mix well, and let it stand overnight. The upper layer is the acetonitrile-saturated n-hexane solution.

Sample Preparation

Solid samples

Use a high-speed pulverizer to pulverize sample, sieving to make the particle size smaller than 2mm aperture, mix evenly and subpackage to 100g in the sample bottle, sealed and preserved for testing.

Liquid samples

Mixing well all the liquid samples in a container by the homogenizer, take 100g (mL) sample to be tested.

Semi-liquid sample

Mashing and mixing with a tissue masher, sealed in the sample bottle and preserved for testing.

Sample Extraction

- **Solid Sample**

  Whole cow milk powder, whole goat milk powder, formula food for infant and young children (0-36 months): such as 1st/ 2nd/ 3rd stage goat milk powder, 1st/ 2nd/ 3rd stage milk powder, etc., milk powder for special dietary uses: such as pregnant and lying-in women milk powder, etc.

  Semi-liquid sample

  Cheese

1. Weigh 1.00 g sample into the 50 mL centrifuge tube.
2. Add 4 mL water to dissolve.
3. Add 2 mL acetonitrile, vortex to mix, and shake in an ultrasonic/vortex shaker for 20 min (or homogenize with the homogenizer for 3 min).
4. Centrifuge for 10 min at ⩾6000 r/min (or filter with glass fiber filter paper after homogenization, except for grease) to obtain the supernatant for preparation of sample solution.

- **Semi-liquid sample**

  Cream

1. Weigh 1.00 g sample into the 50 mL centrifuge tube.
2. Add 4 mL acetonitrile-saturated n-hexane solution.
3. Add 2 mL acetonitrile, vortex to mix, and shake in an ultrasonic/vortex shaker for 20 min (or homogenize with the homogenizer for 3 min).

4. Centrifuge for 10 min at ⩾6000 r/min (or filter with glass fiber filter paper after homogenization, except for grease) to obtain the acetonitrile layer (bottom layer) for preparation of sample solution.

➢ **Liquid Sample**

Liquid milk samples, such as fresh raw cows' milk, fresh raw goats' milk, pure milk, etc.

1. Weigh 4.00 g sample into the 50 mL centrifuge tube.
2. Add 4 mL water to dissolve.
3. Add 2 mL acetonitrile, vortex to mix, and shake in an ultrasonic/vortex shaker for 20 min (or homogenize with the homogenizer for 3 min).
4. Centrifuge for 10 min at ⩾6000 r/min (or filter with glass fiber filter paper after homogenization, except for grease) to obtain the supernatant for preparation of sample solution.

Sample Solution Preparation

1. Accurately pipette all solution obtained in the last step.
2. Add water to 50 mL.
3. Centrifuge at ⩾6000 r/min for 10 min to obtain all of the supernatant as sample solution.

Sample Solution Purification

1. Return the immunoaffinity column to room temperature before use, connect the syringe barrel or loading tube to the immunoaffinity column, and completely dripped out the original liquid in the column.
2. Add 10 mL PBS to the syringe barrel or loading tube to rinse the immunoaffinity column, and completely dripped out the liquid in the column.
3. Accurately pipette the sample solution into the syringe barrel or loading tube, let the sample solution drip at a rate of 1-2 drops per second under gravity pressure.
4. After the sample solution drop is finished, add 20mL water to the syringe barrel or loading tube to rinse the immunoaffinity column. Use a vacuum pump to drain the immunoaffinity column after the drop is finished.
5. Remove the syringe barrel or loading tube, place a 4 mL centrifuge tube under the immunoaffinity column.
6. Add 2 mL methanol to elute the immunoaffinity column. Use a vacuum pump to drain the immunoaffinity column after the drop is finished.

   【*Note: If there is no liquid dripping out about 1 min after adding methanol, please use a syringe to give a little pressure to make the 1- 2 drops of liquid be dripped out, continue to drip out under gravity pressure.*】
7. Collect all the eluates and mix well.
8. After the eluent is blown to near dryness with nitrogen, reconstitute with 1 mL initial mobile phase of HPLC or LS-MC, filter with 0.22 μm microporous filter, and transfer to sample bottle for testing.

Result Interpretation

The content of AFM1 in 2 mL of eluent is equivalent to the content of AFM1 in 1 g of the sample (Liquid milk sample is equivalent to 4 g)

AFM1 content = Detection Concentration × Dilution Factor

Precautions

1. The entire analysis operation should be carried out in the required area. The area should be a relatively independent operating table and waste storage device, and keep away from direct sunlight.
2. During the entire experiment, the operator should take corresponding protective measures in accordance with the requirements for exposure to highly toxic substances.
3. Take out the required number of immunoaffinity columns before use and return them to room temperature.
4. Do not use the immunoaffinity column after the expiration date.
5. The amount of sample to be weighed can be appropriately increased or decreased according to needs, and the amount of sodium chloride and extracting solution can be increased or decreased proportionally.
6. When the content of the toxin in the sample divided by the dilution factor is higher than the column capacity, it is necessary to retest. The customers can appropriately reduce the volume of the sample solution or increase the dilution factor.
7. The optimum pH of the sample solution is between 7-8, check the pH by the pH meter (or pH test strip) before dropping the sample solution into the column. If the pH isn't within this

range, adjust the pH with sodium hydroxide or hydrochloric acid.

8. It is recommended to soak the used container and mycotoxin solution with 5% sodium hypochlorite solution (V/V) overnight.

Validation of Column Capacity and Column Recovery

1. Add 300 ng AFM1 standard stock solution into 30 mL of PBS, and mix well to obtain sample solution.
2. Take three immunoaffinity columns from the same batch. The volume of the added sample is 10 mL (equivalent to 100 ng AFM1).
3. The procedure of adding sample, rinse and elution is the same as the step in "Sample Solution Purification". Rinse with water.
4. Detection and analysis.

The result interpretation:

The result of AFM1 is ⩾80 ng show that the recovery rate is ⩾80%. The product is valid.

Shenzhen Bioeasy Biotechnology Co., Ltd.
ADD: Bioeasy Building 101 (whole building), 1st Liuxian Street, Xingdong Community, Xin'an Road, Bao'an, 518101 Shenzhen, Guangdong Province, PEOPLE'S REPUBLIC OF CHINA
TEL: +86-4001111126 /+86-755-27948546 **FAX:** +86-755-27948417 **Email:** info@bioeasy.com **Web:** www.bioeasy.com