BIOEASY

Immunoaffinity Column for T-2 toxin

Order code: YRIAC3006-1C

Introduction

T-2 toxin is one of the A-type trichothecenes (TS) produced by Fusarium. The fungi that can produce T-2 toxin are mainly soil fungi and some important plant pathogens. T-2 toxins are widely found in nature and can easily contaminate crops and feed. Among them, corn, oats, wheat, barley, and other grain crops are most seriously polluted, which will cause a series of potential food safety risks. In 1973, T-2 toxin was identified by the Food and Agriculture Organization of the United Nations (FAQ) and the World Health Organization (WHO) as one of the most dangerous sources of naturally occurring food contamination.

T-2 toxin is very stable in chemical properties and highly toxic. Accidental ingestion of contaminated grains or feeds by humans and animals will cause a variety of acute and chronic poisoning. The main toxic effects are the digestive system and liver toxicity, cytotoxicity, blood system toxicity, immune system toxicity, reproductive and developmental toxicity, neurotoxicity, etc.

Principle

Based on the specific binding of antibody and antigen, the monoclonal antibodies of T-2 toxin 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 T-2 toxin 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 T-2 toxin 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 T-2 toxin.

After the sample solution is purified by the immunoaffinity column, it can be directly used for qualitative and quantitative detection by LC-MS or derivatized for HPLC, 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

Grain such as corn, wheat, sorghum, etc.

Liquid Sample

Soy sauce, vinegar, liquor, beer, 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: 2000ng

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
6. High-speed homogenizer: 6500 r/min-24000r/min
7. Centrifuge: speed⩾6000 r/min
8. Solid Phase Extraction device (with a vacuum pump)

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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. 1M sodium hydroxide: Weigh 4.00g sodium hydroxide, dissolve in water, and dilute to 100mL.
3. Methanol-aqueous solution (80+20): Add 200 mL water to 800 mL methanol, mix well.
4. Methanol-aqueous solution (16+84): Add 840 mL water to 160 mL methanol, mix well.

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. Alcohol samples need to be ultrasonically degassed first.

Sample Extraction

- **Solid Sample**

Grain such as corn, wheat, sorghum, etc.

1. Weigh 5.00 g sample into the 50 mL centrifuge tube.
2. Add 20 mL methanol-aqueous solution (80+20), vortex to mix, and shake in an ultrasonic/vortex shaker for 20 min (or homogenize with the homogenizer for 3 min).
3. 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.

- **Liquid Sample**

Soy sauce, vinegar, etc.

1. Weigh 25.00 g sample into the 50 mL centrifuge tube.
2. Dilute to 50 mL with methanol, vortex to mix, and shake in an ultrasonic/vortex shaker for 20 min (or homogenize with the homogenizer for 3 min).
3. 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.

- **Liquid Sample**

Liquor, beer, etc.

1. Weigh 20.00 g sample into the 50 mL centrifuge tube.
2. Dilute to 50 mL with methanol, vortex to mix, and shake in an ultrasonic/vortex shaker for 20 min (or homogenize with the homogenizer for 3 min).
3. 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 10mL supernatant.
2. Add 40mL water and mix well.
3. Centrifuge at ⩾6000 r/min for 10 min to obtain 10mL supernatant as sample solution.

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【Note: When the sample is such as vinegar, please use 1M sodium hydroxide to adjust the pH of the sample solution to 7.4】

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. 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.
3. After the sample solution drop is finished, add 10mL 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.

   【Note: If the immunoaffinity column adsorbs more impurities, it can be rinsed with 10 mL of 1% Tween-20 first, and the volume of 1% Tween-20 can be appropriately increased according to the adsorption situation, and finally rinsed with 10 mL of water.】
4. Remove the syringe barrel or loading tube, place a 4 mL centrifuge tube under the immunoaffinity column.
5. Add 1 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.】
6. Collect all the eluates and mix well.
7. 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. Or undiluted or properly diluted eluent, filter with 0.22 μm microporous filter, and transfer to sample bottle for testing.

Result Interpretation

The content of T-2 toxin in 1 mL of eluent is equivalent to the content of T-2 toxin in 0.5 g of the solid sample (Soy sauce and vinegar samples are equivalent to 1g, liquor and beer samples are equivalent to 0.8g)

T-2 toxin 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 6000 ng T-2 toxin standard stock solution into 30 mL of methanol-aqueous solution (16+84), 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 2000 ng T-2 toxin).
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.

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The result interpretation:

The result of T-2 toxin is ⩾1600 ng show that the recovery rate is ⩾80%. The product is valid.

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