BIOEASY

Immunoaffinity Column for Zearalenone (ZEN)

Order code: YRIAC3003-1C

Introduction

Zearalenone (Zearalenone, ZEN), also known as F-2 toxin, is mainly composed of 2,4-dihydroxybenzoic acid lactone compounds produced by Fusarium graminearum and Fusarium culmorum. ZEN mainly pollutes corn, wheat, cereals, and other crops. It has a strong estrogenic effect, which can cause hyperestrogenism and severe reproductive tract symptoms, and infertility. It also has immunotoxicity and genotoxicity.

Principle

Based on the specific binding of antibody and antigen, the monoclonal antibodies of ZEN 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 ZEN 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 ZEN 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 ZEN.

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

Wheat, wheat flour, cornflake, corn, soybean, coix seed, etc.

Liquid Sample

Sesame oil, corn oil, peanut oil, blended oil, soy sauce, vinegar, liquors, etc.

Semi-liquid sample

Soybean paste, 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)
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

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

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. 1% Tween-20: Take 1mL tween-20, dilute to 100mL with water, and mix well.
5. 1M sodium hydroxide: Weigh 4.00g sodium hydroxide, dissolve in water, and dilute to 100mL.
6. 1M hydrochloric acid: Take 8.5mL hydrochloric acid, dilute to 91.5mL with water, and mix well.
7. 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.
8. 1% Tween-20 in PBS (PBST): Take 10mL Tween-20 and dilute to 1000mL with PBS.
9. Acetonitrile aqueous solution (80+20): Add 200 mL water to 800 mL acetonitrile, mix well.
10. Acetonitrile aqueous solution (10+90): Add 900 mL water to 100 mL acetonitrile, 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.

Semi-liquid sample

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

Sample Extraction

- **Solid Sample**

  Wheat, wheat flour, cornflake, corn, soybean, coix seed, etc.

  Liquid Sample

  Corn oil, etc.

  Semi-liquid sample

  Soybean paste, etc.

1. Weigh 5.00 g sample into the 50 mL centrifuge tube.
2. Add 25 mL acetonitrile 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**

  Sesame oil, peanut oil, blended oil, etc.

1. Weigh 5.00 g sample into the 50 mL centrifuge tube.
2. Add 3.00 g NaCl.
3. Add 25 mL acetonitrile 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).
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.

- **Liquid Sample**

  Soy sauce, vinegar, liquors, etc.

1. Weigh 5.00 g sample into the 50 mL centrifuge tube.

2. Dilute to 25 mL with acetonitrile, vortex to mix.
3. Add 3.00 g NaCl. Mix well 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

- **Solid Sample**

  Wheat, wheat flour, cornflake, corn, soybean, coix seed, etc.

  Liquid Sample

  Sesame oil, corn oil, peanut oil, blended oil, soy sauce, vinegar, liquors, etc.

  Semi-liquid sample

  Soybean paste, etc.

1. Accurately pipette 5mL supernatant.
2. Add 35mL ultrapure water and mix well.
3. Centrifuge at ⩾6000 r/min for 10 min to obtain all supernatant as sample solution.

【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. 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.

- **Solid Sample**

  Wheat, wheat flour, cornflake, corn, soybean, coix seed, etc.

  Liquid Sample

  Corn oil, soy sauce, vinegar, liquors, etc.

  Semi-liquid sample

  Soybean paste, etc.

  1) 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.

- **Liquid sample**

  Sesame oil, peanut oil, blended oil, etc.

  1) After the sample solution drop is finished, add 10mL PBS to the syringe barrel or loading tube to rinse the immunoaffinity column. Then add 10mL water 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 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.】
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 ZEN in 2 mL of eluent is equivalent to the content of ZEN in 1 g of the sample

ZEN content = Detection Concentration × Dilution Factor

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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 ZEN standard stock solution into 60 mL of Acetonitrile aqueous solution (10+90), and mix well to obtain sample solution.
2. Take three immunoaffinity columns from the same batch. The volume of the added sample is 20 mL (equivalent to 2000 ng ZEN).
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 ZEN is ⩾1600 ng show that the recovery rate is ⩾80%. The product is valid.

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