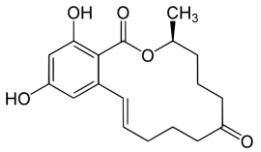
Manual

*aokin*ImmunoClean Immunoaffinity columns for the quantification of Zearalenone



Zearalenone

1.1. General information

The methods listed in this manual are intended for customers with HPLC systems. The ***aokin***ImmunoClean ***ZON*** columns can be used with AOAC Official Methods

aokinImmunoClean columns have been tested and optimized for quantitative measurement of Zearalenone (ZEA, ZON, Zeara) in wheat and other grains.

They may also be used for testing in cereal products and animal feed. For all questions relating to the optimal use of our columns, please contact our experienced technical staff who will be glad to assist you (info@aokin.com).
aokinImmunoClean ***ZON*** columns are used for quantification of Zearalenone in food and feed.

To measure Zearalenone levels, samples are prepared by mixing with an extraction solution, followed by blending, diluting and filtering. The extract is then applied to the ***aokin***ImmunoClean ZON column. The columns contain specific antibodies. The mycotoxin binds to the antibody on the column. The column is then washed to remove impurities of the sample. By passing methanol through the column, the antibody gets denatured and toxin is released. The sample can then be injected into an HPLC system.

1.2. Zearalenone

Zearalenone (ZON) is a mycotoxin which is produced by several *Fusarium species*. It is heat-stable and is found worldwide in a number of cereal crops, such as maize, barley, oats, wheat, rice, and sorghum and also in bread. It has an estrogenic effect and as a primary toxin can cause infertility and abortion and is also known to influence tumor formation of hormone-sensitive tissues such as breast cancer. In line with various regulatory laws, it is required to control the ZON content in food and feed.

1.3. Limitations, shelf life and storage

This product has been designed for use with the protocol and reagents described on the following pages. Do not use materials beyond the expiration date. Deviation from these instructions may not yield optimum results. Do not freeze columns or reagents. Do not keep them in the heat. Store at 2-8°C. It is recommended that reagents should be at ambient temperature for usage, best at 18-22°C.

1.4. General recommendation

- Perform test from beginning to end without interruptions.
- Load sample on column immediately
- Mix the eluate in the cuvette very well before injecting eluate into HPLC.
- Avoid contact of any test reagents or solutions (such as acetonitrile, methanol or column eluate) with rubber or soft flexible plastic. These materials may leach fluorescence into the sample.
- Maintain a slow and steady flow rate through the ***aokin***ImmunoClean ***ZON*** column (1-2 drops/second) during sample loading.
- Elute the column with an incubation step of 3 minutes and at a rate of 1 drop for every 2-3 seconds.

1.5. Types of columns

				
Column type	wide	wide bore	slim	spin
Order No.:	**IC-C+-01-25**	**IC-C-01-25**	**IC-CF-01-25**	**IC-M-01-50**
Package size:	25 units / pack	25 units / pack	25 units / pack	50 units / pack
Elution volume	3mL = 1mL + 2mL	3mL = 1mL + 2mL	3mL = 1mL + 2mL	500µL = 200µL + 300µL
Recommended loading:	< 250 ng	< 500 ng	< 250 ng	< 100 ng

Use of adapters and reservoirs for loading recommended (Order no.: LB-08-13)

1.6. Preparation

1.6.1. Cleaning

All equipment has to be clean and not contaminated with materials that might cause interference with the analysis. All equipment should be washed with a mild detergent solution and then rinsed thoroughly with purified water. This includes glass ware, adapters and syringe barrels used for sample reservoirs. In between assays it is sufficient to rinse with methanol and water. This helps to prevent cross-contamination of samples.

1.6.2. Preparation of reagents

Prepare solutions every week or as needed.

Extraction solvent: Methanol/PBS

Use Methanol HPLC grade only. Use 700 mL methanol and 300 mL PBS buffer, mix.

Diluting buffer: PBS
8.0 g NaCl, 1.2 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.2 g KCl, dissolve in approximately 990 mL purified water, adjust pH to 7.0 with concentrated HCl, bring to 1 liter with purified water.

Wash buffer: PBS/Methanol
Use 100 mL methanol and 900 mL PBS, mix.

Methanol for elution
Use HPLC Grade methanol only.

HPLC Mobile Phases
Acetonitrile:Water:Methanol (45:45:10)
HPLC Grade Acetonitrile: 450 mL
HPLC Grade Methanol: 100 mL
Purified Water: 450 mL
Total Volume: 1000 mL

Methanol:0.01 M Acedic Acid (3:1)
HPLC Grade Methanol: 750 mL
0.01 M Acedic Acid: 250 mL
Total Volume: 1000mL

Water:Methanol:Acetonitrile (40:30:30)
HPLC Grade Acetonitrile: 300 mL
HPLC Grade Methanol: 300 mL
Purified Water: 400 mL
Total Volume: 1000 mL

Solutions should be filtered and degassed before use.

Prepare Working Solutions of Zearalenone (5 µg/mL):
100 µL of Zearalenone Standard (50 µg/mL)
900 µl Acetonitrile
Total Volume: 1 mL

Prepare Zearalenone spiked sample at 250 µg/kg
Add 100 µL Zearalenone Standard (50 µg/mL) sample to 20 g sample

1.7. Materials required for the sample preparation and the HPLC

aokinImmunoClean C+ ***ZON***	(IC-C+-01-25, 25 units/pack)
aokinImmunoClean CF ***ZON***	(IC-CF-01-25, 25 units/pack)
aokinImmunoClean M ***ZON***	(IC-M-01-50, 50 units/pack)
aokin Filter Paper	(LB-05-07-100, 100 units/pack)
Glass fiber filters GF/F	(LB-04-13-GF/F-100, 100 units/pack)
Collection tubes 2 mL	(LB-05-05, 500 units/pack)
Collection tubes 15 mL	(LB-05-01-100, 100 units/pack)
Collection tubes 50 mL	(LB-03-02-1000, 1L)
Methanol, HPLC Grade	
Sodium Chloride, pure	
Acetonitrile, HPLC Grade	
Distilled, reverse osmosis or deionized water	
Graduated Cylinder, 50 mL	(LB-08-16, 1 unit)
Graduated Cylinder, 250 mL	(LB-08-17, 1 unit)
Digital Scale	(LB-07-04, 1 unit)
Commercial Blender, with metal beaker for use with acetonitrile mixtures	
Commercial Blender, with plastic beaker (200 mL) for use with methanol mixtures	(EX-07-06, 1 unit)
Wash Bottle, 500 mL	
Cuvette Rack	
Pump Stand with Air Pump	
Vacuum pump	(LB-04-10, 1 unit)
Vacuum manifold	(LB-04-09, 1 unit)
Filter Funnel, 65 mm	(LB-06-01, 1 unit)
Adjustable Micro-pipettor, 1000 µL	(LB-04-05-1000, 1 unit)
Micro-pipette Tips for adjustable Micro-pipettor, 1000 µL	(LB-04-08-1000L, 100 units/pack)

1.8. Set up and equilibration of columns

Allow column to be at ambient temperature. Remove bottom cap and place the column onto a vacuum manifold, or in a pump stand or collection tube. Open top cap and fill column with wash buffer. Connect adapter and a reservoir to the column. Use a flow rate of 1 mL/min and have 1-2 ml pass through the column. This step ensures an equilibration of the column. Close the valve again to stop the flow.

2. Points of critical importance for reproducibility and recovery

2.1. Representative sampling

A representative sample is essential for accurate and reliable results. Samples should be collected and ground before taking a subsample. Contamination of mycotoxin may differ significantly within a single batch and from kernel to kernel.

2.2. Sample preparation

Different procedures require different reagents. Please make sure that your protocol consists of the following points:

- Adjust to neutral pH.
- Remove all precipitation by glassfiber filtration using a 1.7 µm mesh size.
- Equilibrate column to room temperature, best by rinsing with wash buffer.
- Load column with flow rate of 1 mL/min.
- Wash column with wash buffer
- Dry column by vacuum or air pressure.
- 1 mL Apply Methanol. Incubate for 3 minutes by stopping flow. Apply 2 mL Methanol
- Elute by vacuum or air pressure at 1 mL/minute or by back flushing with a syringe.
- Quantify the concentration by comparing the sample peak height or area to the standard.

aokinImmunoClean ZON columns have been optimized for quantitative measurement of Zearalenone in wheat and corn. Test methods vary in the amount of sample passed through the affinity column resulting in different limits of detection.

General recommendation:

- Perform test from beginning to end without interruptions.
- Load sample on column immediately after dilution.
- Avoid contact of any test reagents or solutions (such as acetonitrile, methanol or column eluate) with rubber or soft flexible plastic. These materials may leach chemicals into the sample.
- Maintain a slow and steady flow rate through the column during sample loading.
- Elute the column slowly, do an incubation step.

Example Procedures:

A1. Standard procedure

Sample extraction:

- Place 50g ground sample with 5 g salt (NaCl) into blender jar.
- Add to jar 100 mL Methanol/PBS buffer (80:20) or alternatively Methanol/water.
- Cover jar and blend at high speed for at least 3.5 minutes.
- Remove cover and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

Dilution:

- Transfer 5 mL filtered extract into another clean vessel.
- Dilute extract 1:7 by adding 30 mL of PBS. Precipitation takes place.
- Check pH to be neutral, if required neutralize by adding small amounts of HCl or NaOH.
- Filter diluted extract through 1.7 µm glass microfibre filter into a clean vessel.

Setup column:

- Connect ***aokin***ICAdapter and a 50 mL syringe barrel (best flow when bubble free).
- Place on vacuum manifold or pump stand.
- Flush with 2 mL wash buffer to ensure equilibration.

Column chromatography:

- Pass 15 mL filtered diluted extract completely through column at a rate of about 1 drops/second until air comes through column.
- Pass 15 mL of wash buffer through the column at a rate of about 2 drops/second.

Dry column with air flow:

- Place new collection tube under column.
- Add 1 mL Methanol.
- Incubate for 3 minutes by stopping flow.
- Pass additional 2 mL Methanol through ***aokin***ImmunoClean at a rate of 1 drop/second. Apply air flow to collect all liquid out of the column.
- Add destilled water to eluate.
- Inject 20 to 100 µl into HPLC.

Alternative procedures in the literature:

Trucksess et al., Journal of AOAC International, 2011, 94 (2), 589 – 595.
Campbell et al., Journal of AOAC International, 2007, 90 (6) 1610 – 1622.
Arranz et al, Journal of AOAC International, 2007, 90 (6), 1598 – 1609.
MacDonald et al. Journal of AOAC International, 2005, 88 (6), 1733 – 1740.
Lombaert et al. Food additives and Contaminants, 2003, 20 (5) 494 – 504.
Llorens et al., Food additives and Contaminants, 2002, 19 (3), 272 – 281.
Kruger et al., Journal of AOAC International, 1999, 82 (6) , 1364 – 1368.
Visconti et al. , Journal of Chromatography A,, 1998, 815, 133 – 140.

B. Setup column

- Connect ***aokin***ICAdapter and a 20 mL syringe barrel (best flow when bubble free).
- Place on vacuum manifold or pump stand.
- Flush with 2 mL wash buffer

C. Recovery

- Recovery of > 80% tested in PBS/Methanol 10 %
- Exact results are found in the attached data sheet.
- Test the recovery of ***aokin***ImmunoClean columns with your protocol and HPLC technique, and use a correction factor as determined.

D. HPLC setup

Example 1:

- Column: reverse phase C18, 4.6 x 75 mm (3 µm)
- Mobile phase: acetonitrile:water (10:90 by volume) degassed, isocratic
- Flow rate: 0.6mL/min
- Injection volume: 50 µL
- Lamp: deuterium or mercury lamp
- Detection: 218 nm
- Sample loop: 200 µL
- Retention time: 5-6 minutes

Example 2:

- Column: reverse phase C18, 3.9 x 300 mm (4 µm) (Waters part #WAT011695)
- Mobile phase: acetonitrile:water (10:90 by volume) degassed, isocratic
- Flow rate: 0.6 mL/min.
- Injection volume: 50 µL
- Lamp: deuterium or mercury lamp
- Detection: 218 nm
- Sample loop: 200 µL
- Retention time: 10-11 minutes

Example 3:

- Column: reverse phase Synergi 4 µm, Hydro-RP, 250 x 4.6 mm, with precolumn, Phenomenex Co.
- Column temperature: 30°C
- Mobile phase: acetonitrile:water (10:90 by volume) degassed, isocratic
- Flow rate: 1.2 mL/min.
- Injection volume: 70 µL
- Detection: 220 nm, Diode array detector
- Retention time: 5.6 – 5.8 minutes

There are a number of equally suitable components that can be used for these examples.

Trouble shooting

3.1. Problem: Samples do not mix

- If samples are very absorbent double the amount of extraction liquid and double the extract volume passed through the column to 20 mL keep the same sensitivity of your analysis system.

3.2. Problem: Overestimation

- Check calculation for spiked sample and standard curve.

3.3. Problem: Underestimation

- Check the extraction procedure.
- Check pH to be neutral before loading the column.
- Control the flow rates and the incubation step for elution.
- Slow down the elution or use a larger amount of elution volume.
- Check calculations for spiked samples.
- Make sure to use the correct HPLC procedure.
- Check calculation for spiked sample and standard curve.
- Control the procedure with analyzing a reference matrix material.

Liabilities

The customer is solely and fully responsible for educating oneself about the proper testing and sampling procedures using this product. ***aokin*** makes no warranty of any kind. ***aokin*** is not liable or responsible for any unsatisfactory or faulty results.

Ordering and technical support

To place an order please contact ***aokin*** at orders@aokin.com

For technical information please contact service@aokin.com

Please contact the application laboratory and service staff for all questions relating to the optimal use of our columns We will be glad to assist you.