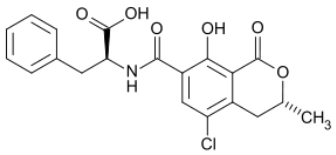
Manual

aokin*ImmunoClean* Immunoaffinity columns for the quantification of Ochratoxin



Ochratoxin A

1.1. General information

aokin*ImmunoClean* ***OTA*** columns are used for quantification of Ochratoxin A (OTA) and Ochratoxin B (OTB) in various sample types.

The methods listed in this manual are intended for customers with HPLC systems.

To measure Ochratoxin levels, samples are prepared by mixing with an extraction solution, followed by blending and filtering. The extract is then applied to the ***aokin****ImmunoClean* ***OTA*** column. The columns contain specific antibodies for Ochratoxin A. 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 Ochratoxin is released. Methanol can then be injected into an HPLC system.

1.2. Ochratoxin

Ochratoxins are mycotoxins which are produced by several *Aspergillus* and *Penicillium species*. Ochratoxins are considered a human carcinogen. Exposure to Ochratoxins through diet can lead to acute toxicity to mammalian kidneys, and may be carcinogenic. In addition Ochratoxins affect the immune system.

In line with various regulatory laws, it is required to control the contamination in food and feed.

1.3. Application

aokin*ImmunoClean* ***OTA*** columns have been tested and optimized for quantitative measurement of Ochratoxin A in wheat, corn, coffee, grapes and wine.

aokin*ImmunoClean* ***OTA*** columns can be used with AOAC Official Methods for the measurement of Ochratoxin in baby food, barley, beer, wine, green coffee and roasted coffee.

They may also be used for testing in licorice baby food, barley, beer, currants, raisins, sultanas, mixed dried fruits, dried figs, ham, rice, meat and urine. For all questions relating to the optimal use of our columns, please contact our technical staff who will be glad to assist you (*info@aokin.com*).

1.4. 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.

1.5. General recommendation

- Perform test from beginning to end without interruptions.
- Load sample on column immediately after centrifugation.
- 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* ***OTA*** column (1-2 drops/second) during sample loading.
- Elute the column at a rate of 1 drop for every 2-3 seconds.

1.6. Types of columns

		
Column type	wide bore	spin
Order No.:	**IC-C-04-25**	**IC-M-04-50**
Package size:	25 units / pack	50 units / pack
Elution volume	3 mL = 1 mL + 2 mL	500 µL = 200 µL + 300 µL
Recommended loading:	< 400 ng	< 50 ng

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

1.7. Preparation

1.7.1. 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.

1.7.2. Preparation of reagents

Prepare solutions every week or as needed.

CAUTION: Methanol, Acetonitrile and the solutions made thereof are flammable. Keep containers in a safe place and thightly capped when not in use.

Extraction solvent: Methanol/1% Sodium bicarbonate (70:30)
Prepare 1 % bicarbonate by taking 3 g $NaHCO_3$ and bring to 300 mL with purified water. Use 700 mL methanol and 300 mL 1% sodium bicarbonate, 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 7.4 with concentrated HCl, bring to 1 liter with purified water.

Wash buffer: PBS/Methanol
Use 5% Tween 20 in PBS and complete the wash step by using PBS.

Methanol/Acetic Acid 1 %
Use 9.9 mL methanol and 100µL glacial acetic acid.

1.8. Materials required for the sample preparation and the HPLC

aokinImmunoClean C ***OTA***	(IC-C-04-25, 25 units/pack)
aokinImmunoClean M ***OTA***	(IC-M-04-50, 50 units/pack)
aokin Filter Paper	(LB-05-10-100, 100 units/pack)
Glass fiber filters GF/F	(LB-04-13-GF/F-100, 100 units/pack)
Reaction tubes (2 ml, with lid)	(LB-05-05, 500 units/pack)
Test tubes (15 ml, with lid)	(LB-05-01-100, 100 units/pack)
Test tubes (50 ml, with lid)	(LB-05-02-250, 250 units/pack)
Methanol, HPLC Grade	(LB-03-02-1000, 1 L)
Sodium Chloride, pure	
Acetonitrile, HPLC Grade	
Distilled, reverse osmosis or deionized water	
Graduated cylinder stand (50 mL)	(LB-08-16, 1 unit)
Graduated cylinder stand (250 mL)	(LB-08-17, 1 unit)
Cuvette Rack	(LB-05-04)
Digital Scale	(LB-07-04, 1 unit)
Commercial blender, with metal or glass beaker for use with Acetonitrile mixtures	(EX-08, 1 unit)
Commercial blender, with plastic beaker (200 mL) for use with Methanol mixtures	(EX-07-06, 1 unit)
Vacuum-pump (diaphragm pump)	(LB-04-10, 1 unit)
Trap for Vacuum-pump (vacuum bottle), 500 mL	(LB-04-12, 1 unit)
Vacuum manifold	(LB-04-09, 1 unit)
Filter funnel (for retainig paper filters)	(LB-06-01, 1 unit)
Adjustable Micropipette, 1000 µL	(LB-04-05-1000, 1 unit)
Micropipette tips for adjustable Micropipette, 1000 µL	(LB-04-08-1000L, 500 units/pack)
aokinReferenceMatrixMaterial ***OTA***	(RMM-04)

1.9. 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 PBS/Methanol (90/10). 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:

- Extract with high solvent ratio.
- Dilute to 10 % max 15 % solvent.
- Adjust to neutral pH.
- Remove all precipitation by glassfiber filtration using a 0.7 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/1%Acetic Acid. Incubate for 3 minutes by stopping flow. Apply 2 mL Methanol/1%Acetic Acid.
- Elute by vacuum or air pressure at 1 mL/minute or by back flushing with a syringe.
- Dilute with purified water.
- Quantify the Ochratoxin concentration by comparing the sample peak height or area to the standard.

aokinImmunoClean ***OTA*** columns have been optimized for quantitative measurement of Ochratoxins in many commodities. 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 centrifugation.
- 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 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. Roasted coffee, green coffee, corn (0 - 50 ppb)

Sample extraction:
- Weigh 25 g ground sample and place in blender jar. Use 200 mL beaker for best blending.
- Add 1.5 g NaCl.
- Add 50 mL Methanol/1% Sodium bicarbonate (70/30).
- Cover beaker and blend at high speed for 3 minutes.
- Remove cover from beaker 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.
- Filter dilute extract through 1.7 µm glass microfibre filter into a clean vessel.

Column Chromatography:
- Pass 20 mL filtered diluted extract trough ***aokin***ImmunoClean column at a rate of about 1mL/minute (about 1 drop/second) until air comes through column.
- Pass 20 mL of Wash buffer through the column at a rate 3 mL/minute.
- Repeat if column bed is dark. Dry column with air flow.
- Place new collection tube under the ***aokin***ImmunoClean column.

Elution:
- Add 1 mL Methanol/1%Acetic Acid.
- Incubate for 3 minutes by stopping flow.
- Pass additional 2 mL Methanol/1%Acetic Acid through ***aokin***ImmunoClean at a rate of 1 drop/second.
- Centrifuge eluate at 15.000 g to remove precipitation or alternatively add 1.5 mL water to dissolve precipitation.

Recovery: > 89 - 105 %

A2. Raisins, other dry fruits (0 - 160 ppb)

Sample extraction:
- Homogenize sample by mixing 500 g sample with 300 mL PBS in a large beaker.
- Use 80 g of this slurry and place it in a 200 mL container of a blender. Add 140 mL Methanol and 30 mL PBS.
- Cover blender jar and blend at high speed for 3 minute.
- Remove cover from jar 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.
- Filter dilute extract through 1.7 µm glass microfibre filter into a clean vessel.

Column Chromatography:
- Pass 10 mL filtered diluted extract trough ***aokin***ImmunoClean column at a rate of about 1mL/minute (about 1 drop/second) until air comes through column.
- Pass 15 mL of Wash buffer through the column at a rate 3 mL/minute.
- Repeat if column bed is dark. Dry column with air flow.
- Place new collection tube under the ***aokin***ImmunoClean column.

Elution:
- Add 1 mL Methanol/1%Acetic Acid.
- Incubate for 3 minutes by stopping flow.
- Pass additional 2 mL Methanol/1%Acetic Acid through ***aokin***ImmunoClean at a rate of 1 drop/second.
- Centrifuge eluate at 15.000 g to remove precipitation or alternatively add 1.5 mL water to dissolve precipitation.

Recovery: > 75 %

A3. Beer

Degas
- Degas beer by vacuum or sonic bath for 30 to 60 minutes.

Sample extraction:
- Adjust pH by adding 2 M NaOH.

Column Chromatography:
- Pass 150 mL sample trough ***aokin****ImmunoClean* column at a rate of about 1mL/minute (about 1 drop/second) until air comes through column.
- Pass 20 mL of 5% Tween in PBS wash buffer followed by PBS through the column at a rate 3 mL/minute.
- Dry column with air flow.
- Place new collection tube under the ***aokin****ImmunoClean* column.

Elution:
- Add 1 mL Methanol/1%Acetic Acid.
- Incubate for 3 minutes by stopping flow.
- Pass additional 2 mL Methanol/1%Acetic Acid through ***aokin****ImmunoClean* at a rate of 1 drop/second.
- Centrifuge eluate at 15.000 g to remove precipitation or alternatively add 1.5 mL water to dissolve precipitation.

Recovery: > 85 %

A4. Wine

Degas
- Degas beer by vacuum or sonic bath for 30 to 60 minutes.

Sample extraction:
- Adjust pH by adding 2 M NaOH.

Column Chromatography:
- Pass 150 mL sample trough ***aokin****ImmunoClean* column at a rate of about 1mL/minute (about 1 drop/second) until air comes through column.
- Pass 20 mL of 5% Tween in PBS wash buffer followed by PBS through the column at a rate 3 mL/minute.
- Dry column with air flow.
- Place new collection tube under the ***aokin****ImmunoClean* column.

Elution:
- Add 1 mL Methanol/1%Acetic Acid.
- Incubate for 3 minutes by stopping flow.
- Pass additional 2 mL Methanol/1%Acetic Acid through ***aokin****ImmunoClean* at a rate of 1 drop/second.
- Centrifuge eluate at 15.000 g to remove precipitation or alternatively add 1.5 mL water to dissolve precipitation.

Recovery: > 85 %

B. 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 PBS to ensure equilibration.

C. Recovery

- Recovery of 80% Ochratoxin tested in PBS buffer.
- 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: 4 µm, 3.9 x 150 mm C18 column Nova-Pak (Waters WAT086344)
- Mobile phase: acetonitrile:water:acetic acid (49.5 : 49.5 : 1 by volume)
- Flow rate: 0.8 mL/min
- Injection volume: 30 - 200 µL
- Fluorescence detector: Waters 474 Scanning Fluorescence Detector
- Detection wavelength: 333 nm excitation and 477 nm emission

Example 2

- Column: 4 µm, 3.9 x 150 mm C18 column Nova-Pak (Waters WAT086344)
- Mobile phase: acetonitrile:water:acetic acid (49.5 : 49.5 : 1 by volume)
- Flow rate: 0.8 mL/min
- Injection volume: 30 - 200 µL
- Fluorescence detector: Waters 474 Scanning Fluorescence Detector
- Detection wavelength: 333 nm excitation and 477 nm emission

Example 3

- Column: Spherisorb ODS 2, 4.6 X 250mm, 5µm (Waters PSS831915), preceded by a C18, 3.9 X 20 mm guard column
- Mobile Phase: water:acetonitrile:acetic acid (49.5:49.5:1 by volume)
- Injection volume: 100 µL
- Flow rate: 1.0 mL/min
- Fluorescence detector: Waters 2475, excitation = 333nm, emission = 477nm
- Column temperature: 25ºC
- Retention time: approximately 11 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 of Ochratoxin

- Check calculation for spiked sample and standard curve.

3.3. Problem: Underestimation of Ochratoxin

- Check the extraction procedure.
- Make sure you use the correct dilution rate. Check pH to be neutral before loading the column.
- Control the flow rates and the incubation step for elution.
- 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.