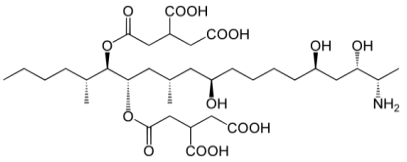
Manual

ImmunoClean Immunoaffinity columns for the quantification of Fumonisin



Fumonisin B1

1.1. General information

ImmunoClean ***FUM*** columns are used for quantification of Fumonisin B1 (FUM) in corn, corn products, food and feed.

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

To measure Fumonisin levels, samples are prepared by mixing with an extraction solution, followed by blending and filtering and diluting. The extract is then applied to the *ImmunoClean* ***FUM*** 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 elution solvent through the column, the antibody gets denatured and Toxin is released. Methanol can then be injected into an HPLC system.

1.2. Fumonisin

Fumonisin B1 (FUM) is a mycotoxin which is produced by several *Aspergillus* and *Penicillium species*. Fumonisin B1 is the most common and most important mycotoxin of Fumonisins. It is an inhibitor of ceramide synthase and disrupts the biosynthesis of membranes. Fumonisin B1 is hepatotoxic and nephrotoxic in all animal species tested. In line with various regulatory laws, it is required to control the contamination in food and feed.

1.3. Application

ImmunoClean ***FUM*** columns have been tested and optimized for quantitative measurement of Fumonisin in corn, corn products and mixtures.

ImmunoClean ***FUM*** columns can be used with AOAC Official Methods for the measurement.

They may also be used for testing in cereal products and animal feed.

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
- 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 *ImmunoClean* ***FUM*** 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	wide bore	slim	spin
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:	< 500 ng	< 300 ng	< 300 ng	< 100 ng

Use of adapters and reservoirs for loading recommended

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/Water
Use Methanol HPLC grade only. Use 800 mL methanol and 200 mL deionized water.

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

Diluting Buffer: Bicarbonate solution
2.5 g NaCl, 0.5 g $NaHCO_3$ bring to 100 mL with deionized water.

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 /1 % acetic acid for elution
Use HPLC Grade methanol only.
Use 99 mL methanol and 1 mL acetic acid, mix.

1.8. Materials required for the sample preparation and the HPLC

ImmunoClean C+ ***FUM***
ImmunoClean CF ***FUM***
ImmunoClean M ***FUM***
Filter Paper
Glass fiber filters GF/F
Collection tubes 2 mL
Collection tubes 15 mL
Collection tubes 50 mL
Methanol, HPLC Grade
Sodium Chloride, pure
Acetonitrile, HPLC Grade
Sodium bicarbonate
Glacial acetic acid, 99% purity
Distilled, reverse osmosis or deionized water
Graduated Cylinder, 50 mL
Graduated Cylinder, 250 mL
Digital Scale
Commercial Blender, with metal beaker for use with acetonitrile mixtures
Commercial Blender, with plastic beaker (200 mL) for use with methanol mixtures
Wash Bottle, 500 mL
Cuvette Rack
Pump Stand with Air Pump
Vacuum pump
Vacuum manifold
Filter Funnel, 65 mm
Adjustable Micro-pipettor, 1000 µL
Micro-pipette Tips for adjustable Micro-pipettor, 1000 µL

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. 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:

- Dilute filtrate to 10 % solvent ratio
- 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 PBS.
- Load column with flow rate of 1 mL/min.
- Wash column with PBS, deionized water is not recommended.
- 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.
- Quantify the concentration by comparing the sample peak height or area to the standard.

ImmunoClean ***FUM*** columns have been optimized for quantitative measurement of Fumonisins 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 filtration.
- 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. Grain and corn

Sample extraction:

- Weigh 50 g ground sample and place in blender jar. Add 5 g NaCl. Use 200 mL beaker for best blending.
- Add 100 mL Methanol/water (80/20) or Methanol/buffer (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.
- Transfer 10 mL filtered extract into another clean vessel.
- Dilute extract with 40 mL PBS. Mix well.
- Check pH to be neutral, if required neutralize.
- Use a glass fiber filtration through 1.7 µm glass microfibre filter into a clean vessel.

B. Set up column

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

Column Chromatography:

- Pass 10 mL filtered diluted extract trough *ImmunoClean* column at a rate of about 1mL/minute (about 1 drop/second) until air comes through column.
- Pass 10 mL of PBS 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 *ImmunoClean* column.

Elution:

- Add 1 mL Methanol/1%Acetic Acid Incubate for 5 minutes by stopping flow.
- Pass additional 2 mL Methanol/1%Acetic Acid through *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.

C. Recovery

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

D. HPLC setup

Derivatization and fluorescence detection or alternative mass spectrometer detection recommended.

A2. Beer

Sample extraction:

- Use 5 mL beer

B. Set up column

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

Column Chromatography:

- Pass 5 mL beer trough *ImmunoClean* column at a rate of about 1mL/minute (about 1 drop/second) until air comes through column.
- Pass 10 mL of Bicarbonate solution through the column at a rate 3 mL/minute.
- Dry column with air flow.
- Place new collection tube under the *ImmunoClean* column.

Elution:

- Add 1 mL Methanol/1%Acetic Acid. Incubate for 5 minutes by stopping flow.
- Pass additional 2 mL Methanol/1%Acetic Acid through *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.

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- Recovery of > 80% tested in PBS buffer.
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D. HPLC setup

Derivatization and fluorescence detection or alternative mass spectrometer detection recommended.

Liabilities

The customer is solely and fully responsible for educating oneself about the proper testing and sampling procedures using this product.

Methods and references

AOAC method 2001.04 Determination of Fuminisin B1 and B2 in Corn and Cornflakes by Liquid Chromatography and Immunoaffinity Column Clean Up J. of AOAC Int. 84(6) 1828-1837, 2001

CEN 14352 Determination of Fumonisin B1 and B2 in maize based foods – HPLC method with immunoaffinity column clean up.

CEN 16187 Determination of Fumonisin B1 and B2 in processed maize containing foods for infants and young children – HPLC method with immunoaffinity column cleanup and fluorescence detection after precolumn derivatization.

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.