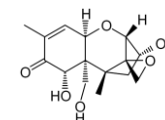




Manual

ImmunoClean Immunoaffinity columns for the quantification of Deoxynivalenol



Deoxynivalenol

1.1. General information

ImmunoClean **DON** columns are used for quantification of Deoxynivalenol DON in grain.

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

To measure Deoxynivalenol levels, samples are prepared by mixing with an extraction solution, followed by blending and filtering. The extract is then applied to the *ImmunoClean* **DON** 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 Deoxynivalenol is released. Methanol can then be injected into an HPLC system.

1.2. Deoxynivalenol

Deoxynivalenol (DON) is a mycotoxin which is produced by several *Fusarium* species. It interferes at the ribosomal level by inhibiting DNA, RNA and protein synthesis. DON has a haemolytic effect of erythrocytes. In acute poisoning, vomiting occurs. It is suspected of causing cancer.

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

1.3. Application

ImmunoClean **DON** columns have been tested and optimized for quantitative measurement of Deoxynivalenol in wheat and other grains.

ImmunoClean **DON** columns can be used with AOAC Official Methods for the measurement of Deoxynivalenol.

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* **DON** 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	< 500 ng	< 500 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 and acetonitrile and the solutions made thereof are flammable. Keep containers in a safe place and tightly capped when not in use.

Extraction solvent: PBS / PEG

8.0 g NaCl, 1.2 g Na₂HPO₄, 0.2 g KH₂PO₄, 0.2 g KCl, 50 g PEG 8000 dissolve in approximately 900 mL purified water, adjust pH to 7.0 with concentrated HCl, add PEG bring to 1 liter with purified water.

Alternatively use 250mM Phosphate buffered saline

40 g NaCl, 6 g Na₂HPO₄, 1 g KH₂PO₄, 1 g KCl, dissolve in approximately 900 mL purified water, adjust pH to 7.0 with concentrated HCl, bring to 1 liter with purified water.

Methanol

Use HPLC grade only

Preparation of working solutions

DON working solution 1 (10µg/mL): 50µl of DON standard (200µg/mL) + 950 µl deionized water.

DON working solution 2 (1µg/mL): 100µl of DON working solution 1 (10µg/mL) + 900 µl deionized water.

Preparation of DON standards

0.1 ppm (µg/g) X 0.25 g sample equivalent = 0.025 µg
 0.025 µg ÷ 1µg/mL (DON working solution 2) = 0.025mL = 25µl
 Add 25 µl DON working solution 2 to 975µl methanol

0.5 ppm (µg/g) X 0.25 g sample equivalent = 0.125 µg
 0.125 µg ÷ 1µg/mL (DON working solution 2) = 0.125mL = 125µl
 Add 125 µl DON working solution 2 to 875µl methanol

5 ppm (µg/g) X 0.25 g sample equivalent = 1.25 µg
 1.25 µg ÷ 10µg/mL (DON working solution 1) = 0.125mL = 125µl
 Add 125 µl DON working solution 1 to 875µl methanol



1.8. Materials required for the sample preparation and the HPLC

ImmunoClean C+ DON
ImmunoClean CF DON
ImmunoClean M DON
Filter Paper
Glass fiber filters GF/F
Reaction tubes (2 ml, with lid)
Test tubes (15 ml, with lid)
Test tubes (50 ml, with lid)
Methanol, HPLC Grade
Sodium Chloride, pure
Acetonitrile, HPLC Grade
Distilled, reverse osmosis or deionized water
Graduated cylinder stand (50mL)
Graduated cylinder stand (250mL)
Cuvette Rack
Digital Scale
Commercial blender, with metal or glass beaker for use with
Acetonitrile mixtures
Commercial blender, with plastic beaker (200 mL) for use with
Methanol mixtures
Vacuum-pump (diaphragm pump)
Trap for Vacuum-pump (vacuum bottle), 500 mL
Vacuum manifold
Filter funnel (for retaining paper filters)
Adjustable Micropipette, 1000 µL
Micropipette tips for adjustable Micropipette, 1000 µL
ReferenceMatrixMaterial DON

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:

- 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, distilled water is not recommended.
- 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.

ImmunoClean DON columns have been optimized for quantitative measurement of Deoxynivalenol 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.
- 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

Sample extraction:

- Weigh 25 g ground sample and place in blender jar. Use 200 mL beaker for best blending.
- Add 100 mL PBS
- Cover beaker and blend at high speed for 1 to 3 minutes.
- Remove cover from beaker and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
- Check pH to be neutral, if required neutralize.
- If filtrate is very cloudy use a glass fiber filtration through 1.7 µm glass microfibre filter into a clean vessel.

Column Chromatography:

- Pass an exact amount (10 to 20 mL) filtered diluted extract through *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
- Incubate for 5 minutes by stopping flow.
- Pass additional 2 mL Methanol 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.

Recovery: > 89 - 105 %

B. Setup 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

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

HPLC Conditions 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

HPLC Conditions 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



HPLC Conditions 3:

- Column: reverse phase Synergi 4 μ m, 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 μ L
- 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

- **Do not use deionized water for loading or washing, use PBS/PEG or if PEG is to be avoided use alternatively 250 mM PBS**
- Check the extraction procedure.
- 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.