IMMUNOPREP® ONLINE DEOXYNIVALENOL

Product Code: P902/48

Online immunoaffinity cartridges for use in conjunction with a RIDA®CREST system.
For *in vitro* use only.

P902/V3/13.06.19

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R-BIOPHARM
RHÔNE LTD

Contents

Test Principle

The online deoxynivalenol immunoaffinity cartridge is used in conjunction with the RIDA®CREST system, combining automated online sample application with quantitative analysis of deoxynivalenol. The immunoaffinity cartridge contains a monoclonal antibody that is specific for deoxynivalenol coupled to a hydrophilic polymer that can withstand high pressure. This enables the cartridge to be incorporated directly online with the RIDA®CREST system.

The immunoaffinity cartridge offers highly specific, sensitive, rapid and automated analysis for deoxynivalenol in a wide range of food and feed matrices. Using the deoxynivalenol immunoaffinity cartridge, the sample application, washing and elution are performed online for a specified number of analyses before the cartridge is automatically removed and replaced with a new cartridge. This level of reuse has been found to offer optimum cartridge performance and prevent interference or carryover.

Following extraction of the toxin from the sample with solvent, the extract is filtered, diluted and transferred to an autosampler vial. The diluted extract is injected onto the immunoaffinity cartridge and any toxin present in the sample is retained by antibody in the cartridge. Unbound material is then removed by washing the cartridge and sending the resulting wash to waste. Subsequently the toxin are released from the antibody following online elution and the complete eluate from the cartridge is quantitatively analysed for total deoxynivalenol by HPLC.

Reagents Not Provided

- Distilled / Deionised Water (suitable for use with LC, e.g. MilliQ)
- Ammonium acetate (pro analysis grade)
- Tris (hydroxymethyl) aminomethane (>99.9%, ultra quality)
- Acetic Acid (glacial, LC grade)
- Hydrochloric acid
- Sodium Hydroxide
- Solvents (LC Grade Methanol)
- Deoxynivalenol Standard (please refer to Preparation of Standards section)

Accessory Products

- Glass Microfiber Filter Paper
- Nylon, 0.22 um, diameter 25 mm, syringe membrane filters
- 40 cm PEEK™ tubing (orange label) with an internal diameter of 0.25 mm*

*available from R-Biopharm, please contact your local distributor for further information.

Cartridge Handling

Please refer to the Cartridge Handling Instructions included in the kit for details on how to handle the cartridges and store them for short periods of time.

Note: IMMUNOPREP® ONLINE DEOXYNIVALENOL cartridges must not be allowed to sit in position in the tray without buffer for more than 24 hours to prevent the antibody drying out. It is essential to run a standard through every cartridge on each day for correct calibration of samples.

Recommended Methods and Application Notes

Methods are available for all matrices covered by legislation as well as additional commodities. Deviation from the methods described in our Instructions For Use may not result in optimum results. However, it is possible as part of the validation process that R-Biopharm Rhône can support customer specific methods. Please contact your local R-Biopharm distributor for further information.

Hazards

Mycotoxins are very hazardous substances. Only laboratories equipped to handle toxic materials and solvents should perform analyses. Suitable protective clothing, including gloves, safety glasses and lab coats should be worn throughout the analysis.

Flammable solvents should be stored in an explosion-proof cabinet. Use a chemical hood and protective equipment as applicable.

Contact your local R-Biopharm distributor for a Material Safety Data Sheet for further information if required.

Decontamination

Prior to disposal, excess standard solutions should be treated with at least one-tenth their volume of 5 % sodium hypochlorite. Labware and contaminated waste should be immersed in 5 % sodium hypochlorite solution for 30 minutes followed by the addition of 5 % acetone for 30 minutes. Flush with copious amounts of water before disposal. After decontamination labware should be thoroughly washed. Incinerate waste if regulations permit.

Storage & Shelf Life

The cartridges have an expiry of 12 months from date of manufacture if stored at 2 – 8 °C in buffer. It is advised when the cartridges are not in use for periods of more than 24 hours then they should be stored in the buffer supplied at 2 – 8 °C. This will ensure optimum shelf life and keep the immunoaffinity packing in the cartridge hydrated. Do not freeze. For further information please refer to the Cartridge Handling Instructions.

It is important to note that the antibody included in the immunoaffinity cartridge can be denatured by extreme temperature or pH change.

Sampling

A representative sample should be obtained by following one of the officially recognised sampling procedures. It is recommended that a minimum of 1 kg of representative sample is finely ground and a portion (10 - 50 g dependent on method used) of this is removed and extracted.

Sensitivity

The sensitivity is dependent on the final detection system employed by the analyst.

For optimal cartridge performance, aim to load sample containing a quantity of 1.25 ng up to 250 ng of deoxynivalenol onto the cartridge. Do not exceed the quantity of 250 ng as this is close to the capacity of the cartridge.

Recoveries

In general recoveries of greater than 80 % of deoxynivalenol are achieved providing the injected amount of toxin stays within the binding capacity (1.25 ng to 250 ng). Please note the capacity decreases if higher flow rates are used during sample loading. For highly contaminated samples (deoxynivalenol content in final extract greater than 250 ng/ml), it is recommended to further dilute the extract with the appropriate dilution buffer.

Recommended Re-Usability

It is essential to run a standard through a cartridge each day of analysis for correct calibration of samples and to correct for recovery. To offer optimum cartridge performance and to reduce the chance of interference or carryover we would recommended to inject a blank (i.e. water), standard, 12 test samples and then another standard (for bracketed calibration) through each cartridge (a total of 15 injections).

Cartridge Preparation

Cartridges should be at ambient temperature before use. Prior to use, the antibody is activated by conditioning the cartridge with loading buffer. This can be automatically programmed as part of the sample clean-up program.

Preparation of Buffers

When preparing buffers it is important to ensure that they are within the pH range specified.

• Preparation of Loading Buffer (100 mM Ammonium Acetate*)

1. Add 1 litre of water to a flask.
2. Add 7.7 g of ammonium acetate.
3. Adjust the pH to 6.8 - 7.0.

• Preparation of Cartridge Wash Buffer (150 mM Ammonium Acetate* containing 20 mM Tris)

1. Add 1 litre of water to a flask.
2. Add 11.55 g of ammonium acetate.
3. Add 2.4 g of tris (hydroxymethyl) aminomethane.
4. Adjust the pH to 6.8 - 7.0 using concentrated hydrochloric acid.

• Preparation of Elution Buffer (50 % Methanol containing 20 mM Ammonium Acetate*)

1. Add 500 ml of water and 500 ml of methanol to a flask.
2. Add 1.54 g of ammonium acetate.
3. Adjust the pH to 6.8 - 7.0.
4. Degas the buffer in a sonic bath for 30 minutes.

***Note**: in case of a fluctuating baseline, which may affect the LOD/LOQ, it is possible to replace ammonium acetate in the loading buffer, wash buffer and elution buffer with lithium acetate (LC-MS/MS grade).

• Mobile Phase A (2 % Methanol containing 0.02 % Acetic Acid)

1. Add 20 ml of methanol and 200 µl of acetic acid to a flask.
2. Make up to 1 litre with water.
3. De-gas in a sonic bath for 30 minutes.

• Mobile Phase B (90 % Methanol containing 0.02 % acetic acid)

1. Add 900 ml of methanol and 200 µl of acetic acid to a flask.
2. Make up to 1 litre with water.
3. De-gas in a sonic bath for 30 minutes.

• Autosampler Wash (50 % Methanol)

• Pump Seal Wash Solution (20 % Isopropanol)

Sample Preparation

• Cereal and cereal based foods

1. Weigh 5 g of ground sample into a 50 ml flask or a 1 litre capacity, solvent resistant blender jar.
2. Add 20 ml of water and allow the sample to absorb the water for 15 minutes.
3. Shake for 45 minutes or blend at high speed for 2 minutes.
4. Centrifuge at 4,000 rpm for 10 minutes.
5. Filter the supernatant through glass microfibre filter paper.
6. Dilute 5 ml of filtrate with 5 ml of water.
7. Adjust pH to 7.5 - 8.0 using 0.2 M sodium hydroxide.
8. Transfer 1 ml of the diluted filtrate over a 0.2 µm syringe filter and collect in an amber autosampler vial.
9. Inject 250 µl onto the RIDA®CREST system.

• Feed

1. Weigh 5 g of ground sample into a 50 ml flask or a 1 litre capacity, solvent resistant blender jar.
2. Add 20 ml of water and allow the sample to absorb for 15 minutes.
3. Shake for 45 minutes or blend at high speed for 2 minutes.
4. Centrifuge at 4,000 rpm for 10 minutes.
5. Filter the sample through glass microfibre filter paper.
6. Dilute 5 ml of filtrate with 20 ml of water.
7. Adjust pH to 7.5 - 8.0 using 0.2 M sodium hydroxide.
8. Transfer 1 ml of the diluted filtrate over a 0.2 µm syringe filter and collect in an amber autosampler vial.
9. Inject 250 µl onto the RIDA®CREST system.

Preparation of Standards

Preparation of 100,000 ng/ml deoxynivalenol stock solutions:

1. Ready-to-use Deoxynivalenol (100,000 ng/ml) is available from Sigma Aldrich.

Calibration Standard

The diluted standard solution should be prepared fresh on the day of analysis and used within a 24 hour period. It is essential to run a standard through every cartridge on each day for correct calibration of samples.

Examples of how to prepare calibration standards (can be modified according to legislative requirements or contamination levels):

- **For Routine Analysis**

Low level standard (i.e. 500 ng/ml deoxynivalenol):

1. Take 50 µl of 100 µg/ml deoxynivalenol standard and make up to 10 ml with water.
2. Inject 250 µl of standard 1 onto the RIDA®CREST system.

High level standard (i.e. 1,000 ng/ml deoxynivalenol):

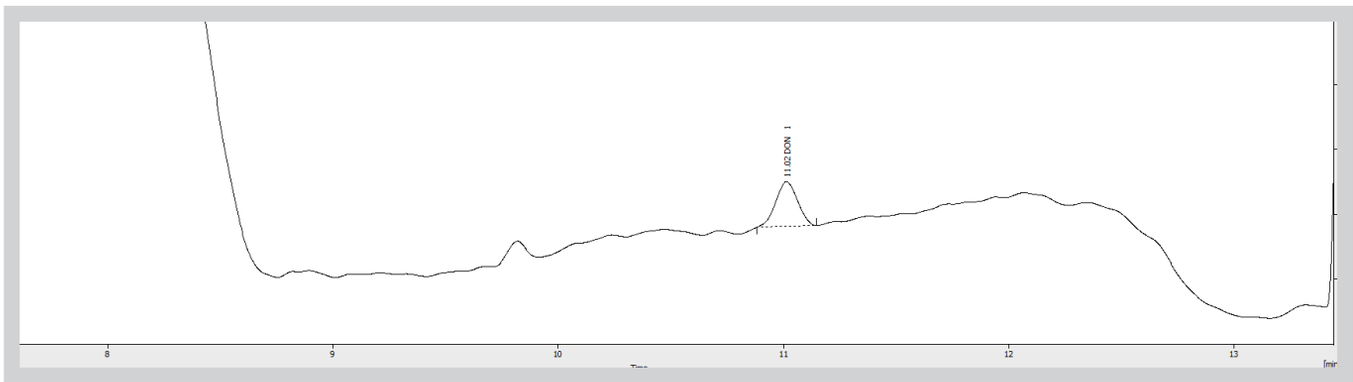
1. Take 100 µl of 100 µg/ml deoxynivalenol standard and make up to 10 ml with water.
2. Inject 250 µl of standard 1 onto the RIDA®CREST system.

Recommended RIDA®CREST Conditions

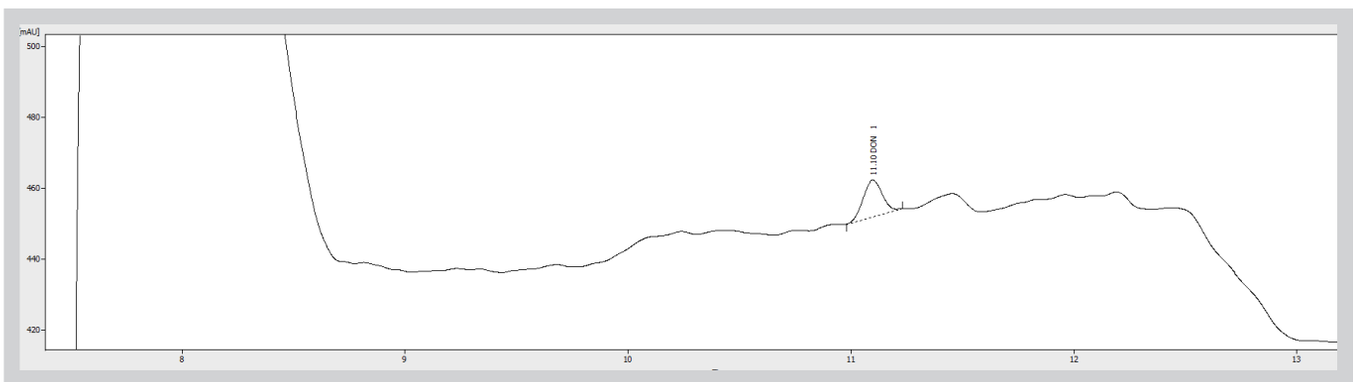
RIDA®CREST Conditions				
Analytical Column	InertSustain AQ C18 5 µm, 4.6 mm x 250 mm or equivalent			
Connection between ACE and analytical column	40 cm of PEEK™ tubing with an internal diameter of 0.25 mm.			
HPLC Pump 1 (Line A1)	Mobile Phase A. Please refer to Preparation of Buffers section.			
HPLC Pump 2 (Line B1)	Mobile Phase B. Please refer to Preparation of Buffers section.			
Gradient HPLC	Time (min)	% A1	% B1	Flow Rate (ml/min)
	Initial	100	0	1.1
	3.00	100	0	1.1
	4.00	100	0	1.2
	5.00	80	20	1.2
	10.00	50	50	1.2
	12.00	50	50	1.2
	12.10	0	100	1.3
	14.00	0	100	1.3
	14.01	100	0	1.3
	16.00	100	0	1.3
HPD1 (Line 1A)	Loading Buffer. Please refer to Preparation of Loading Buffer section.			
HPD1 (Line 1B)	Wash Buffer. Please refer to Preparation of Wash Buffer section.			
HPD1 (Line 1C)	Elution Buffer. Please refer to Preparation of Elution Buffer section.			
Recommended RIDA®CREST Conditions for Sample Clean-Up	Conditioning	HPD flow 5,000 µl/min, volume 2,000 µl of loading buffer.		
	Sample Extract	HPD flow 100 µl/min, volume 250 µl of loading buffer.		
	Cartridge Wash	HPD flow 667 µl/min, volume 2,000 µl of wash buffer.		
	Elution	HPD flow 100 µl/min, volume 400 µl of elution buffer.		
	Clamp Wash	HPD flow 1,000 µl/min, volume 2,000 µl of loading buffer.		
UV-VIS Detector	Excitation: 220 nm			
Column Heater	Maintain guard and analytical columns at 45 °C			
Data Control System	Clarity or from preferred supplier			
Injection Volume	Inject 250 µl onto the RIDA®CREST system.			

Example HPLC Chromatograms

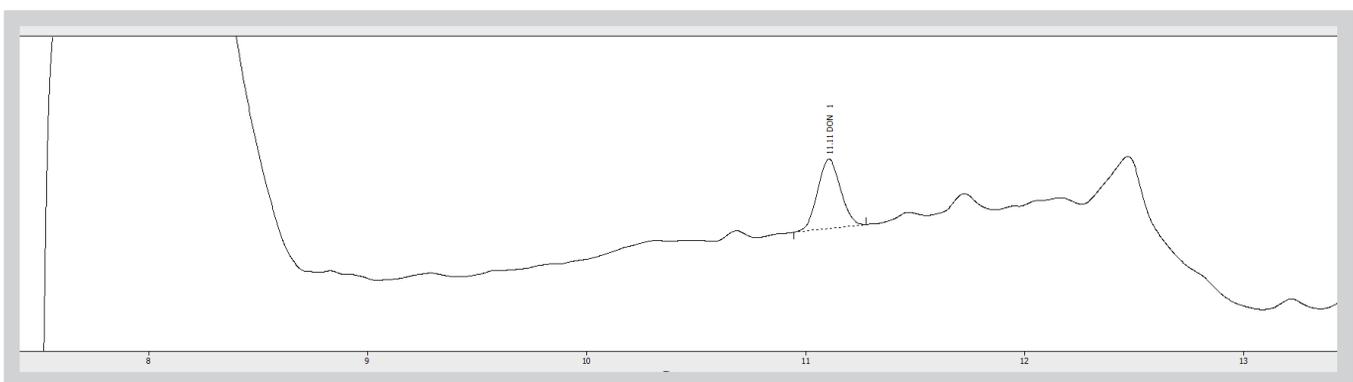
- **Example HPLC Chromatogram for Wheat (Spiked at 750 ppb)**



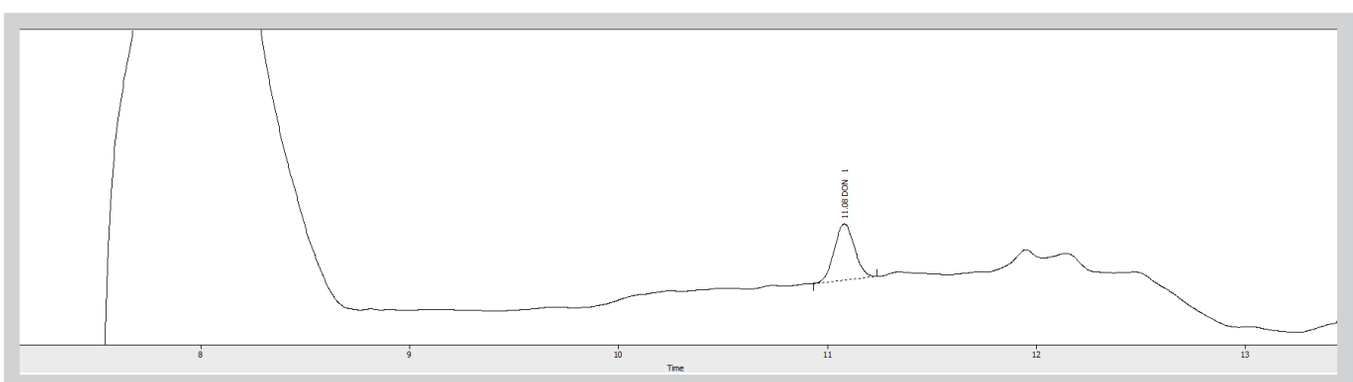
- **Example HPLC Chromatogram for Maize (Spiked at 750 ppb)**



- **Example HPLC Chromatogram for Ruminant Feed (Spiked at 5,000 ppb)**



- **Example HPLC Chromatogram for Dried Distillers Grain (Spiked at 5,000 ppb)**



Quality

RBR products are developed, manufactured, tested and dispatched under an ISO 9001 registered Quality Management System, guaranteeing a consistent product, which always meets our performance specifications. Our products have been used in many collaborative studies to develop standard European and International Methods and are widely used by key institutions, food companies and government laboratories. Customer references for RBR products are available on request.

Technical Support

RBR understand that from time to time users of our products may need assistance or advice. Therefore, we are pleased to offer the following services to our customers:

- Analysis of problem samples.
- Application notes for difficult samples.
- References from the RBR library.
- Installation and support of the KOBRA® CELL.
- Advice on detection parameters.
- Advice on preparation and handling of standards.
- Updates on legislation, sampling and other news by e-mail.
- Provision of spiked samples.

Please contact your local R-Biopharm distributor for further information.

Warranty

R-Biopharm Rhône Ltd makes no warranty of any kind, express or implied, except that all products made by R-Biopharm Rhône Ltd are made with materials of suitable quality. If any materials are defective, R-Biopharm Rhône Ltd will provide a replacement product. The user assumes all risk and liability resulting from the use of R-Biopharm Rhône Ltd products and procedures. R-Biopharm Rhône Ltd shall not be liable for any damages, including special or consequential damages, loss or expense arising directly or indirectly from the use of R-Biopharm Rhône Ltd products or procedures.

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