DZT MS-PREP®

Product Code: P73 / P73B

Immunoaffinity columns for use in conjunction with LC-MS/MS.
For in vitro use only.

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RHÔNE LTD

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Test Principle

The procedure is based on monoclonal antibody technology, which makes the test highly specific, sensitive, rapid and simple to perform.

The columns contain a gel suspension of monoclonal antibodies specific to the toxins of interest. Following extraction of the toxins the sample extract is filtered, diluted and passed slowly through the immunoaffinity column. Any toxins which are present in the sample are retained by the antibody within the gel suspension. The column is washed to remove unbound material and the toxins are then released from the column following elution with solvent. The eluate is collected prior to analysis by LC-MS/MS.

The total extraction and clean-up time takes approximately 20 minutes to perform. The result is improved clean-up and concentration of the toxins from food and feed samples reducing ion suppression and removing the need to use matrix matched standards. This provides cleaner chromatography, improved sensitivity and greater accuracy. The columns also have the added advantage that they can be automated for large scale analysis of samples.

Reagents Not Provided

- Distilled / Deionised Water (suitable for use with HPLC, e.g. MilliQ)
- Solvents (Methanol and Acetonitrile)
- Phosphate Buffered Saline (PBS) (RP202)*
- Mycotoxin Standards (Please refer to Preparation of Standards section)
- Ammonium Bicarbonate

Accessory Products

- Whatman No. 113 or No. 4 Filter Paper
- Glass Microfiber Filter Paper
- Immunoaffinity Column Rack (CR1)*
- Immunoaffinity Column Accessory Pack (AP01)*

* Available from R-Biopharm. 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.

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 and Application Notes may not achieve optimum results. Please contact your local R-Biopharm distributor for further information.

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 columns expire 18 months from date of manufacture if stored at 2 - 8 °C or 12 months from date of manufacture if stored at 21 - 25 °C. Do not freeze.

Ensure the column has not dried out and contains buffer above the gel. It is important to note the antibody included in the immunoaffinity column 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 (5 - 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. However the test sensitivity may be improved if required by increasing the volume of sample passed through the immunoaffinity column. Please note the ratio of solvent to phosphate buffered saline (PBS) should be maintained.

Recoveries

If an analyst wishes to account for losses during extraction it is recommended a spiked sample of the same commodity type as the material being tested is analysed following the complete procedure as a reference standard. The recoveries obtained with the spiked sample can be used to correct the results obtained with the test sample.

Column Preparation

Immunoaffinity columns should be at ambient temperature before use. Remove the cap from the top of the column and discard. Firmly attach the column to a glass syringe barrel using an adapter and place in an immunoaffinity column rack or clamp stand.

Elution

In order to fully elute the toxin/s from the immunoaffinity column it is vital that the solvent is in contact with the antibody within the gel suspension for a sufficient period of time. This ensures that all of the bonds between the antibody and the toxin are broken, ultimately releasing all of the toxin from the column for analysis with the detection system of choice

To ensure that the solvent is in contact with the antibody gel for a sufficient period of time any of the following elution methods can be used: -

Backflushing (this is the preferred method of choice at R-Biopharm): backflush by gently raising and lowering the syringe plunger during passage of the solvent through the column. This process will reverse the direction of flow of the eluate through the gel. This should be repeated 3 times before collecting the eluate. Proceed to the next step in the method.

Application of small volumes of solvent: apply the volume of solvent required for elution in two or three smaller aliquots. Allow each aliquot to remain in contact with the gel suspension for a minimum of 30 seconds before allowing each to pass fully through the gel suspension for collection. Proceed to the next step in the method.

Incubation with solvent: apply the full volume of solvent required for elution and allow 2-3 drops of the solvent to pass through the column for collection. Allow the remainder of the solvent to remain in contact with the gel suspension for a minimum of 60 seconds before allowing it to pass through the gel suspension for collection. Proceed to the next step in the method.

Sample Preparation

• Cereal

This method has been tested on a number of cereals including wheat, barley, maize and cereal based products such as biscuits and pasta.

1. Weigh 25 g of ground sample into a 1 litre capacity, solvent resistant blender jar.
2. Add 100 ml of 70 % methanol and blend at high speed for 2 minutes.
3. Filter the sample through Whatman No. 113 or No. 4 filter paper, or centrifuge at 4,000 rpm for 10 minutes.
4. Dilute 2 ml of filtrate with 48 ml of phosphate buffered saline (PBS).
5. Filter the diluted extract through glass microfibre filter paper.
6. Pass 20 ml of the filtrate (equivalent to 0.2 g of sample) through the column at a flow rate of 2 ml per minute (or the sample can be allowed to pass through the column by gravity if preferred). A slow, steady flow rate is essential for the capture of the toxin by the antibody.
7. Wash the column by passing 20 ml of water through at a flow rate of approximately 5 ml per minute. Pass air though the column to remove residual liquid.
8. Elute the toxins from the column at a flow rate of 1 drop per second using 1 ml of 100 % methanol and collect in a 5 ml amber glass vial. Please refer to the Elution section for further information.
9. Following elution pass 1 ml of water through the column and collect in the same vial to give a 2 ml total volume.
10. Inject 50 µl onto the LC-MS/MS system.

Preparation of Standards

• Deoxynivalenol Stock Solution

It is advised to start with a 100,000 ng/ml deoxynivalenol stock solution.

• Preparation of Zearalenone Stock Solution

It is advised to start with a 10,000 ng/ml zearalenone stock solution.

• Preparation of T-2 and HT-2 Stock Solution

It is advised to start with a 100,000 ng/ml T-2 or HT-2 toxin stock solution.

Combined Working Standard

- **Deoxynivalenol Standard Dilution**

1. Measure 500 µl of 100 % methanol into an amber vial.
2. Add 500 µl of 100,000 ng/ml deoxynivalenol solution to give a 50,000 ng/ml deoxynivalenol solution.

- **Zearalenone Standard Dilution**

1. Measure 500 µl of 100 % methanol into an amber vial.
2. Add 500 µl of 10,000 ng/ml zearalenone solution to give a 5,000 ng/ml zearalenone solution.

- **T-2 and HT-2 Standard Dilution**

1. Measure 1 ml of 100 % methanol into an amber vial.
2. Remove 400 µl to waste.
3. Add 200 µl of 100,000 ng/ml T-2 solution and 200 µl of 100,000 ng/ml HT-2 solution (equivalent to 20,000 ng/ml T-2 and 20,000 ng/ml HT-2 solution, or 40,000 ng/ml total solution).

Calibration Curve

It is recommended to run at least a 3 - 6 point calibration curve. In constructing a suitable curve the levels of the calibration standards should bracket or include the range of expected results. The diluted standard solutions should be prepared fresh on the day of analysis and used within a 24 hour period.

Example of how to prepare a three point calibration curve (can be modified according to legislative requirements or contamination levels):

1. Mixed Standard:
   - Take 3 ml of 100 % methanol and remove 366 µl to waste.
   - Add 96 µl of 50,000 ng/ml deoxynivalenol standard, 180 µl of 5,000 ng/ml zearalenone standard and 90 µl of 40,000 ng/ml T-2 and HT-2 standard (equivalent to 1,600 ng/ml deoxynivalenol, 300 ng/ml zearalenone and 1,200 ng/ml T-2 and HT-2).
2. Standard 3: Take 500 µl of mixed standard and add 2 ml of 100 % methanol and 2.5 ml of water (equivalent to 160 ng/ml deoxynivalenol, 30 ng/ml zearalenone and 120 ng/ml T-2 and HT-2).
3. Standard 2: Take 2.5 ml of standard 3 and add 2.5 ml of 50 % methanol (equivalent to 80 ng/ml deoxynivalenol, 15 ng/ml zearalenone and 60 ng/ml T-2 and HT-2).
4. Standard 1: Take 2.5 ml of standard 2 and add 2.5 ml of 50 % methanol (equivalent to 40 ng/ml deoxynivalenol, 7.5 ng/ml zearalenone and 30 ng/ml T-2 and HT-2).
5. Inject 50 µl of each solution onto the LC-MS/MS system.

Recommended LC conditions

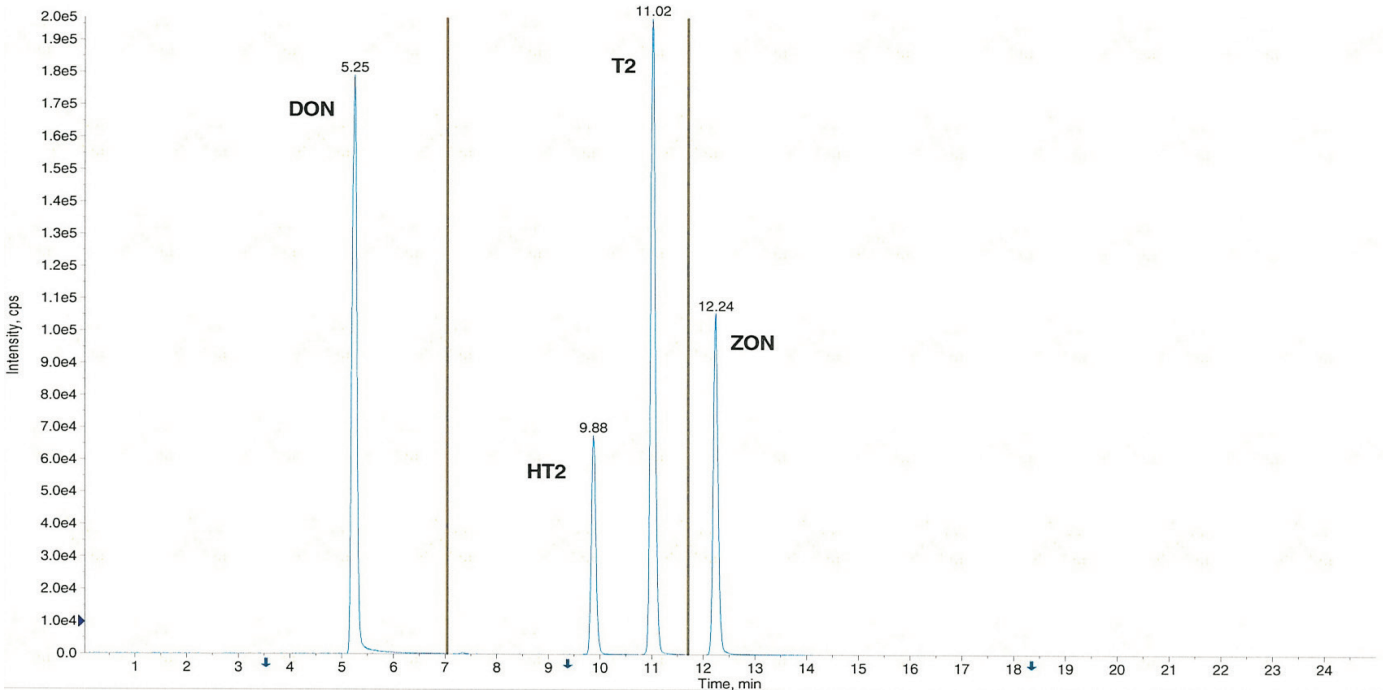
LC Conditions			
Guard Cartridge	Phenomenex Gemini C18 4 mm x 2 mm or equivalent		
Analytical Column	Phenomenex Gemini 5 µm C18 110 A, 150 mm x 3 mm or equivalent		
Mobile Phase	Solution A: Deionised water containing 2 mM ammonium bicarbonate Solution B: Methanol Prepare fresh on day of analysis.		
Gradient Conditions	Time (min)	% Solution A	% Solution B
	0	90	10
	0.1	90	10
	2	50	50
	10	20	80
	15	20	80
	16	90	10
	25	90	10
HPLC Pump	To deliver mobile phase		
Flow Rate	0.3 ml per minute		
Column Heater	Maintain guard and analytical column at 45 °C		
Integrator / Data Control System	From preferred supplier		
Injector	Autosampler / Rheodyne valve		
Injection Volume	50 µl		

Mass Spectrometry Conditions	
Instrument	Applied Biosystems API3000 LC-MS/MS with TurbolonSpray®
Mode	Multiple Reaction Monitoring (MRM) Mode with negative / positive / negative polarity
TurboProbe Temperature	450 °C
TurboProbe Heater Gas (Gas 2)	8 L/min (set manually)
IonSpray Voltage	4500 V (positive or negative)
IonSpray Nebuliser Gas (Gas 1)	10
Curtain Gas	6
Collision Gas	12

Instrument Settings										
Period No. &	Time (min)	Toxin	RT (min)	Q1 Precursor Ion (m/z)	Q3 Product Ion (m/z)	Dwell Time (ms)		Focusing Potential (V)	Collision Energy (V)	Collision Exit Potential (V)
1 Neg	0 - 7	DON	5.2	295.3	**264.9** (Quantifier) 138.2 (Qualifier)	200	-25.6	-150.0	-16.4 -23.9	-6.0 -9.0
2 Pos	7 - 11.7	HT-2	9.9	442.1	**263.1** (Quantifier) 215 (Qualifier)	200	26.5	170.0	17.5 18.7	5.6 20.0
		T-2	11.0	484.2	**305.1** (Quantifier) 245.2 (Qualifier)	200	30.4	190.0	20.7 19.8	28.0 15.0
3 Neg	11.7 - 25	ZON	12.2	317.3	**131.1** (Quantifier) 175.2 (Qualifier)	200	-58.0	-300.0	-40.5 -33.8	-7.1 -12.0

Typical LC-MS/MS Total Ion Count Chromatogram for Analysis of Deoxynivalenol, Zearalenone, T-2 and HT-2 Using DZT MS-PREP® Immunoaffinity Columns

- **Cereal**



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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