IMMUNOPREP® ONLINE OCHRATOXIN

Product Code: P901/48, P901

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

P901/V4/05.10.18

www.r-biopharm.com

R-BIOPHARM
RHÔNE LTD

Contents

Test Principle

The online ochratoxin immunoaffinity cartridge is used in conjunction with the RIDA®CREST system, combining automated online sample application with quantitative analysis of ochratoxin A. The immunoaffinity cartridge contains a monoclonal antibody that is specific for ochratoxin A 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 ochratoxin A in a wide range of food and feed matrices. Using the ochratoxin 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 the antibody in the cartridge. Unbound material is then removed by washing the cartridge and sending the resulting wash to waste. Subsequently the toxin is released from the antibody following online elution with elution buffer and the complete eluate from the cartridge is quantitatively analysed for ochratoxin A by HPLC.

Reagents Not Provided

- Distilled / Deionised Water (suitable for use with LC, e.g. MilliQ)
- Solvents (HPLC Grade Methanol and Acetonitrile)
- Ammonium Acetate
- Sodium Tetraborate Decahydrate
- Triton X-100
- Tween 20
- Sodium Bicarbonate
- Sodium Hydroxide
- Acetic Acid
- Isopropanol
- Ochratoxin A Standard (please refer to Preparation of Standards section)

Accessory Products

- Whatman No. 113 or No. 4 Filter Paper
- Glass Microfibre Filter Paper
- 0.2 µm Nylon Membrane (35 mm diameter) Syringe Filter

* Available from R-Biopharm. Please contact your local R-Biopharm 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 OCHRATOXIN 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. Sodium tetraborate decahydrate is highly toxic and may damage fertility and unborn child. Any steps involving sodium tetraborate decahydrate should be performed in a ventilated fume hood. 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 18 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 0.02 ng up to 0.7 ng (dependent on sensitivity of system used) of ochratoxin A onto the cartridge. Do not exceed the quantity of 0.7 ng as this is close to the capacity of the cartridge.

Recoveries

In general a recovery of greater than 80 % for ochratoxin A is achieved providing the injected amount of toxin stays within the binding capacity (0.02 ng to 0.7 ng). Please note the capacity decreases if higher flow rates are used during sample loading. In addition, the ratio of solvent to dilution buffer should not be increased. For highly contaminated samples (ochratoxin A content in final extract is greater than 0.7 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. 3 % Tween 20 in water), standard, 12 test samples and then another standard (for bracketed calibration) through each cartridge (a total of 15 injections).

Preparation of Buffers

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

• Preparation of Loading Buffer (20 mM Ammonium Acetate)

1. Add 1 litre of water to a flask.
2. Add 1.54 g of ammonium acetate.
3. Adjust the pH to 6.8 - 7.0 using 1 M sodium hydroxide.

• Preparation of Cartridge Wash Buffer (10 % Methanol containing 25 mM Sodium Tetraborate, 20 mM Ammonium Acetate and 0.1 % Triton X-100 (w/v))

1. Add 1 g of Triton X-100 to a flask.
2. Add 900 ml of water.
3. Add 9.53 g of sodium tetraborate decahydrate and 1.54 g of ammonium acetate.
4. Add 100 ml of methanol.
5. Adjust pH to 8.3 - 8.5 using acetic acid.

• Preparation of Elution Buffer (Acetonitrile : Water : Acetic Acid (60 : 33 : 7 v/v/v) containing 20 mM Ammonium Acetate)

1. Add 330 ml of water to a flask.
2. Add 70 ml of acetic acid and 1.54 g of ammonium acetate.
3. Add 600 ml of acetonitrile.

• Mobile Phase A (Acetonitrile : Water : Acetic Acid (48 : 50 : 2 v/v/v))

• Mobile Phase B (Methanol : Acetic Acid : Water (90 : 5 : 5 v/v/v))

• Autosampler Wash Solution (50 % Methanol)

• Pump Seal Wash Solution (20 % Isopropanol)

Sample Preparation

• Wheat

1. Weigh 25 g of ground sample into a 1 litre capacity, solvent resistant blender jar.
2. Add 100 ml of 60 % acetonitrile and blend at high speed for 2 minutes (alternatively sample can be shaken for 30 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 1 ml of filtrate with 9 ml of 3 % Tween 20 in water.
5. Transfer 1.5 ml of the diluted filtrate into an amber autosampler vial.
6. Depending on the sensitivity of the fluorescence detector, inject 0.5 - 1 ml onto the RIDA®CREST system.

• Maize and Green Coffee

1. Weigh 25 g of ground sample into a 1 litre capacity, solvent resistant blender jar.
2. Add 100 ml of acetonitrile : methanol (60 : 40 v/v) and blend at high speed for 2 minutes (alternatively sample can be shaken for 30 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 1 ml of filtrate with 9 ml of 3 % Tween 20 in water.
5. Transfer 1.5 ml of the diluted filtrate into an amber autosampler vial.
6. Depending on the sensitivity of the fluorescence detector, inject 0.5 - 1 ml onto the RIDA®CREST system.

• Figs

1. Weigh 50 g of slurry (i.e. 16.7g of ground sample mixed with 33.3 ml of water) into a 1 litre capacity, solvent resistant blender jar.
2. Add 75 ml of 1 % sodium bicarbonate solution and blend at high speed for 2 minutes (alternatively sample can be shaken for 30 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 1 ml of filtrate with 9 ml of 3 % Tween 20 in water.
5. Filter the diluted extract through glass microfibre filter paper.
6. Transfer 1.5 ml of the diluted filtrate into an amber autosampler vial.
7. Depending on the sensitivity of the fluorescence detector, inject 0.5 - 1 ml onto the RIDA®CREST system.

Sample Preparation

• Paprika

1. Weigh 25 g of dry weight 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 (alternatively sample can be shaken for 30 minutes).
3. Filter the sample through a glass microfibre filter paper.
4. Dilute 0.5 ml of filtrate with 9.5 ml of 3 % Tween 20 in water.
5. Transfer 1.5 ml of the diluted filtrate into an amber autosampler vial.
6. Depending on the sensitivity of the fluorescence detector, inject 0.5 - 1 ml onto the RIDA®CREST system.

• Nutmeg

1. Weigh 25 g of dry weight ground sample and 1 g of sodium bicarbonate into a 1 litre capacity, solvent resistant blender jar.
2. Add 100 ml of acetonitrile : methanol (60 : 40 v/v) and blend at high speed for 2 minutes (alternatively sample can be shaken for 30 minutes).
3. Filter the sample through a glass microfibre filter paper.
4. Dilute 0.5 ml of filtrate with 9.5 ml of 1 % sodium bicarbonate solution.
5. Filter 2 ml of diluted filtrate through a 0.2 µm nylon membrane syringe filter.
6. Transfer 1.5 ml of the filtrate into an amber autosampler vial.
7. Depending on the sensitivity of the fluorescence detector, inject 0.5 - 1 ml onto the RIDA®CREST system.

Preparation of Standards

Preparation of 1,000 ng/ml ochratoxin A stock solutions:

1. Ready-to-use OCHRASTANDARD (P11 / P11A, 1,000 ng/ml) is available from R-Biopharm.

or

1. Alternatively, crystalline powder of ochratoxin A can be purchased. Contact your local R-Biopharm distributor for further information. The ochratoxin powder is reconstituted as per the instructions provided and left overnight in the dark at room temperature to give a stock concentrate.
2. This is then used to prepare a 1,000 ng/ml ochratoxin A stock solution.

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. 3 ppb):

1. Take 50 µl of 1,000 ng ochratoxin standard and make up to 1 ml with acetonitrile : methanol (60 : 40 v/v) (equivalent to 50 ng/ml).
2. Take 150 µl at 50 ng/ml and make up to 10 ml with acetonitrile : methanol (60 : 40 v/v) (equivalent to 0.75 ng/ml).
3. Low Level Standard: Take 1 ml at 0.75 ng/ml and make up to 10 ml with 3 % Tween 20 in water (equivalent to 0.075 ng/ml).
4. Depending on the sensitivity of the fluorescence detector, inject 1 ml of standard 1 onto the RIDA®CREST system.

High level standard (i.e. 30 ppb):

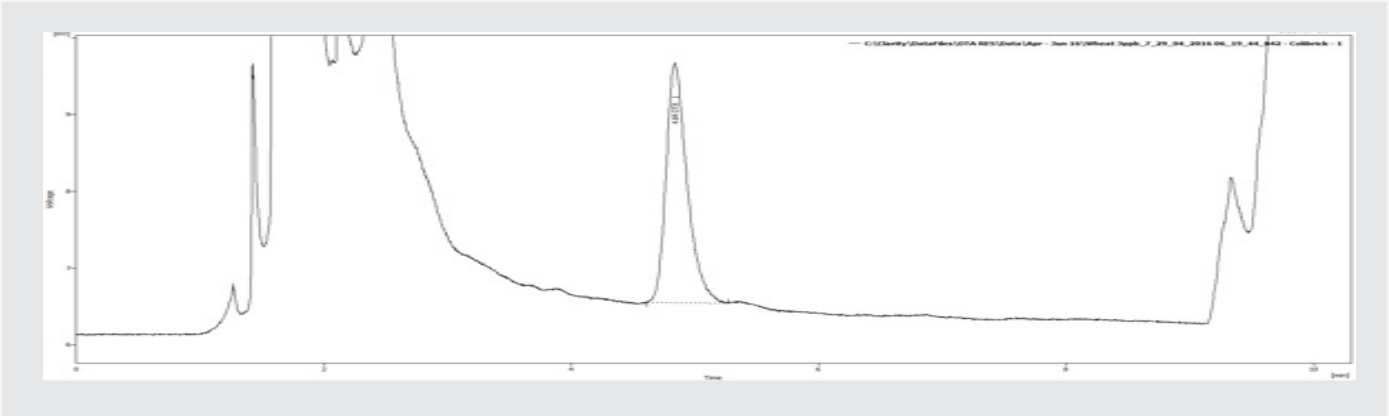
1. Take 50 µl of 1,000 ng ochratoxin standard and make up to 1 ml with acetonitrile : methanol (60 : 40 v/v) (equivalent to 50 ng/ml).
2. High Level Standard: Take 100 µl at 50 ng/ml and make up to 10 ml with 3 % Tween 20 in water (equivalent to 0.5 ng/ml).
3. Depending on the sensitivity of the fluorescence detector, inject 1 ml of standard 1 onto the RIDA®CREST system.

Recommended RIDA®CREST Conditions

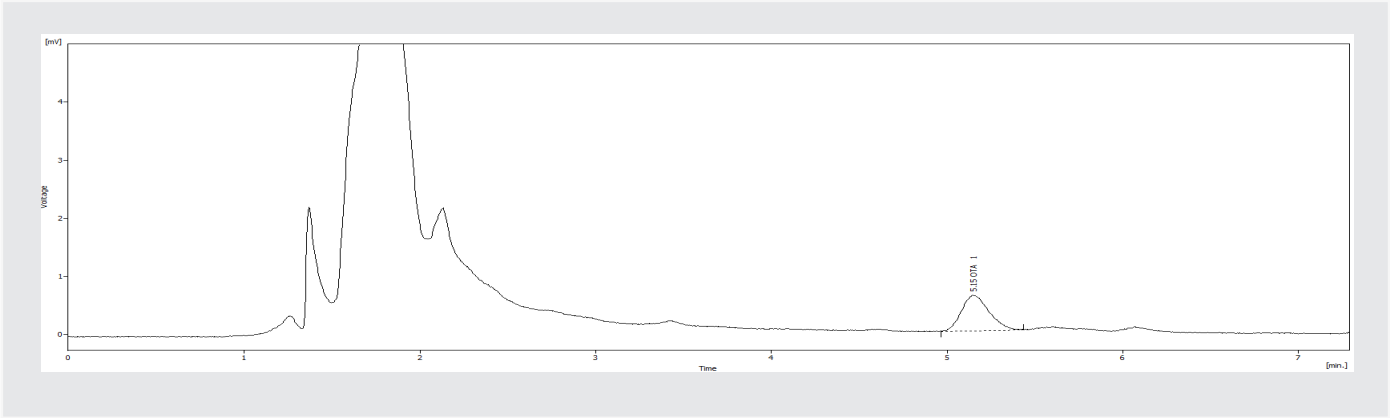
RIDA®CREST Conditions				
Guard Cartridge	Supelco LC-18 5 µm, 4 mm x 10 mm			
Analytical Column	Supelco LC-18 5 µm, 4.6 mm x 150 mm or equivalent			
HPLC Pump 1 (Line A1)	Mobile Phase A Acetonitrile : Water : Acetic Acid (48 : 50 : 2 v/v/v).			
HPLC Pump 2 (Line B1)	Mobile Phase B. Methanol : Acetic Acid : Water (90 : 5 : 5 v/v/v).			
Flow Rate Pump 1	Variable			
Gradient HPLC	Time (min)	% A1	% B1	Flow Rate (ml/min)
	Initial	100	0	1.2
	0.01	100	0	0.6
	0.50	100	0	0.6
	0.55	100	0	1.2
	8.00	100	0	1.2
	8.01	0	100	1.5
	9.00	0	100	1.5
	9.01	100	0	1.2
HPD1 (Line 1A)	Loading Buffer. Please refer to Preparation of Buffers section.			
HPD1 (Line 1B)	Wash Buffer. Please refer to Preparation of Buffers section.			
HPD1 (Line 1C)	Elution Buffer. Please refer to Preparation of Buffers section.			
Recommended RIDA®CREST Conditions for Sample Analysis*	Conditioning	HPD flow 5,000 µl/min, volume 2,000 µl of Loading Buffer		
	Sample Extract	HPD flow 500 µl/min, volume 1,000 µl of Loading Buffer Or HPD flow 250 µl/min, volume 500 µl of Loading Buffer		
	Cartridge Wash	HPD flow 1,000 µl/min, volume 6,000 µl of Cartridge Wash Buffer		
	Elution	HPD flow 600 µl/min, volume 300 µl of Elution Buffer		
	Clamp Wash	HPD flow 5,000 µl/min, volume 2,000 µl of Loading Buffer		
Fluorescence Detector	Excitation: 335 nm			
	Emission: 475 nm			

Example HPLC Chromatograms

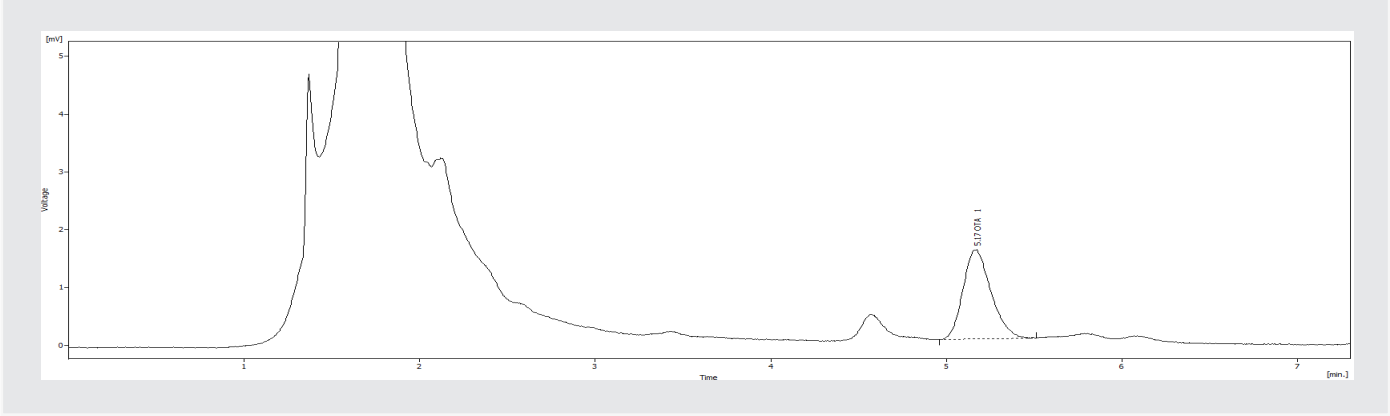
- **Example HPLC Chromatogram for Wheat (Spiked at 3 ppb)**



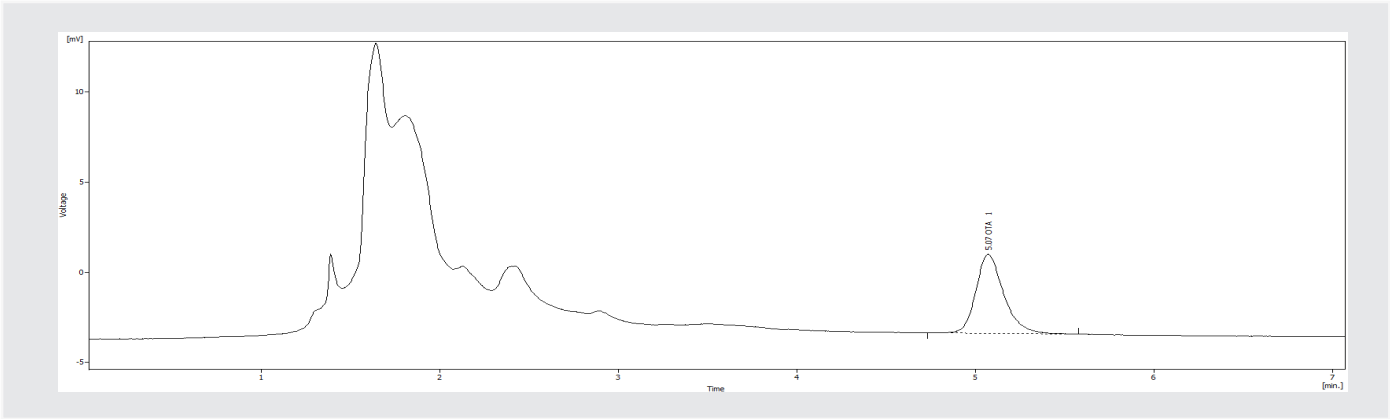
- **Example HPLC Chromatogram for Maize (Spiked at 3 ppb)**



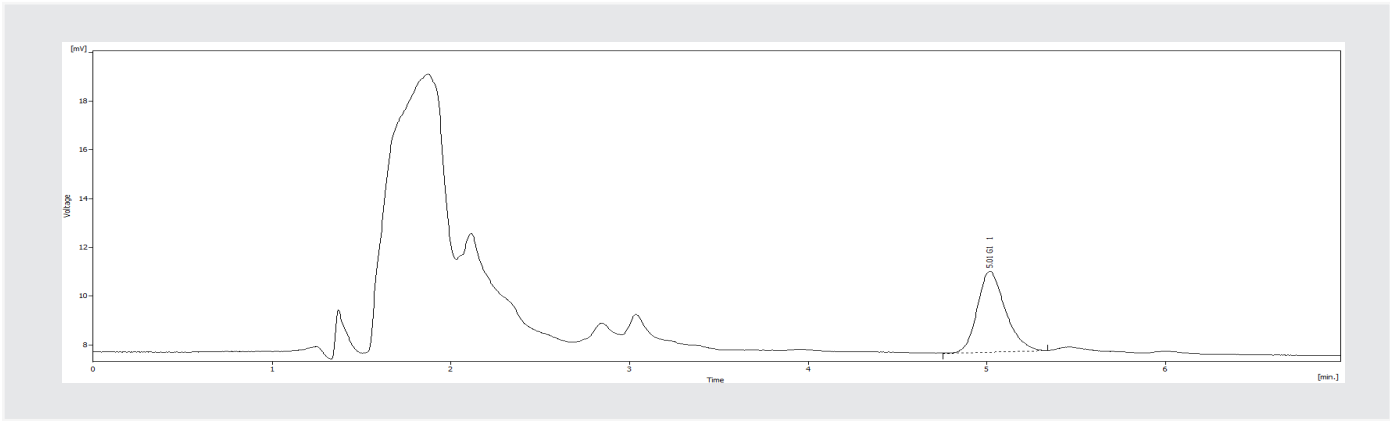
- **Example HPLC Chromatogram for Figs (Spiked at 10 ppb)**



- **Example HPLC Chromatogram for Paprika (Spiked at 30 ppb)**



- **Example HPLC Chromatogram for Nutmeg (Spiked at 30 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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