

AOF MS-PREP[®]

Product Code: P115 / P115B

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

P115/V6/13.12.19

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RHÔNE LTD

Contents

	Page
Test Principle.....	4
Reagents Not Provided.....	4
Accessory Products.....	4
Hazards.....	4
Recommended Methods and Application Notes.....	5
Decontamination.....	5
Storage & Shelf Life.....	5
Sampling.....	5
Sensitivity.....	5
Recoveries.....	5
Column Preparation.....	5
Elution.....	6
Sample Preparation.....	7
• Cereal.....	7
• Animal Feed.....	8
Preparation of Standards.....	9
• Preparation of Total Aflatoxin Stock Concentrate.....	9
• Preparation of Ochratoxin Stock Concentrate.....	9
• Preparation of Fumonisin Stock Concentrate.....	9
Calibration Curve.....	10
Recommended LC Conditions.....	10
Example LC-MS/MS Total Ion Count Chromatogram for Maize.....	12
Example LC-MS/MS Total Ion Count Chromatogram for Animal Feed.....	13
Quality.....	14
Technical Support.....	14
Warranty.....	14

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 is retained by the antibodies 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 (HPLC Grade Methanol and Acetonitrile)
- Phosphate Buffered Saline (PBS) (RP202)*
- Mycotoxin Standards (Please refer to Preparation of Standards section)
- Ammonium Formate
- Formic Acid
- Acetic Acid
- Sodium Chloride
- Sodium Hydroxide (to pH filtrate if required)

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 result in 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 have an expiry of 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 that the column has not dried out and contains buffer above the gel. It is important to note that 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 (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. However the test sensitivity may be improved if required by increasing the volume of sample passed through the immunoaffinity column. Please note that 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 that a spiked sample of the same commodity type as the material being tested be analysed following the complete procedure as a reference standard. The recoveries obtained with the spiked sample can then 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

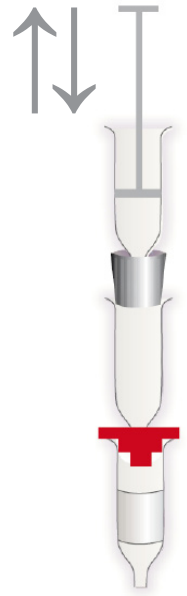
In order to fully elute the toxin/s from the immunoaffinity column it is vital that the solvent is in contact with the antibody within the gel suspension for a sufficient period of time. This ensures that all of the bonds between the antibody and the toxin are broken, ultimately releasing all of the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

1. Weigh 25 g of ground sample and 5 g of sodium chloride into a 1 litre capacity, solvent resistant blender jar.
2. Add 100 ml of 60 % 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 10 ml of the filtrate with 15 ml of phosphate buffered saline (PBS).
5. Filter the diluted extract through glass microfiber filter paper.
6. Pass 5 ml of the filtrate (equivalent to 0.5 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 through 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 an 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 μ l onto the LC-MS/MS system.

Sample Preparation

• Animal Feed

1. Weigh 25 g of ground sample into a 1 litre capacity, solvent resistant blender jar.
2. Add 100 ml of acetonitrile : acetic acid : water (74 : 1 : 25 v/v/v) 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 5 ml of the filtrate with 75 ml of phosphate buffered saline (PBS).
5. Adjust to around pH 7.4 using 2 M sodium hydroxide.
6. Filter the diluted extract through glass microfibre filter paper.
7. Pass 32 ml of the filtrate (equivalent to 0.5 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 toxins by the antibody.
8. Wash the column by passing 20 ml of water through at a flow rate of approximately 5 ml per minute. Pass air through the column to remove residual liquid.
9. Elute the toxins from the column at a flow rate of 1 drop per second using 1 ml of 100 % methanol and collect in an amber glass vial. Please refer to the Elution section for further information.
10. Following elution pass 1 ml of water through the column and collect in the same vial to give a 2 ml total volume.
11. Inject 25 μ l onto the LC-MS/MS system.

Preparation of Standards

• Preparation of Aflatoxin Stock Solutions

Preparation of 1,000 ng/ml aflatoxin B1, B2, G1 and G2 stock solutions:

1. Ready-to-use AFLASTANDARD (P22 / P22A, 1,000 ng/ml) is available from R-Biopharm.

or

1. Alternatively, crystalline powder of aflatoxins B1, B2, G1 and G2 can be purchased. Contact your local R-Biopharm distributor for further information. The 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 aflatoxin B1, B2, G1 and G2 stock solution.

Note: The ratio of B1, B2, G1 and G2 may vary in each standard. Please note the correct ratio for the standard purchased.

• Preparation of Ochratoxin Stock Solutions

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.

• Preparation of Fumonisin Stock Solutions

Preparation of 150,000 ng/ml fumonisin stock solution:

1. Crystalline powder of fumonisin can be purchased. Contact your local R-Biopharm distributor for further information. The 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 150,000 ng/ml fumonisin (FB1 and FB2) stock solution.

Note: The ratio of FB1 to FB2 may vary in each standard. Please note the correct ratio for the standard purchased.

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 five point calibration curve (can be modified according to legislative requirements or contamination levels):

- Standard 5:
 - Take 5 ml of 100 % methanol and remove 200 µl to waste.
 - Add 100 µl of 1,000 ng/ml total aflatoxin standard, 50 µl of 1,000 ng/ml ochratoxin A standard and 100 µl of 150,000 ng/ml fumonisin standard.
 - Add 4.95 ml of water (equivalent to 10 ng/ml total aflatoxin, 5 ng/ml ochratoxin A and 1,500 ng/ml fumonisin).
- Standard 4: Take 5 ml of standard 5 and add 5 ml of 50 % methanol (equivalent to 5 ng/ml total aflatoxin, 2.5 ng/ml ochratoxin A and 750 ng/ml fumonisin).
- Standard 3: Take 4 ml of standard 4 and add 6 ml of 50 % methanol (equivalent to 2 ng/ml total aflatoxin, 1 ng/ml ochratoxin A and 300 ng/ml fumonisin).
- Standard 2: Take 5 ml of standard 3 and add 5 ml of 50 % methanol (equivalent to 1 ng/ml total aflatoxin, 0.5 ng/ml ochratoxin A and 150 ng/ml fumonisin).
- Standard 1: Take 5 ml of standard 2 and add 5 ml of 50 % methanol (equivalent to 0.5 ng/ml total aflatoxin, 0.25 ng/ml ochratoxin A and 75 ng/ml fumonisin).
- Inject 50 µl of each standard 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 : Methanol : Formic Acid (95 : 5 : 0.1 v/v/v) containing 1 mM Ammonium Formate Solution B: Deionised Water : Methanol : Formic Acid (2 : 98 : 0.1 v/v/v) containing 1 mM Ammonium Formate Prepare fresh on day of analysis.		
Gradient Conditions	Time (min)	% Solution A	% Solution B
	0	80	20
	0.1	80	20
	10	10	90
	15	10	90
	15.1	80	20
	25	80	20
HPLC Pump	To deliver mobile phase		
Flow Rate	0.3 ml per minute		
Column Heater	Maintain guard and analytical columns at 40 °C		
Integrator / Data Control System	From preferred supplier		
Injector	Autosampler / Rheodyne valve		
Injection Volume	50 µl or 25 µl		

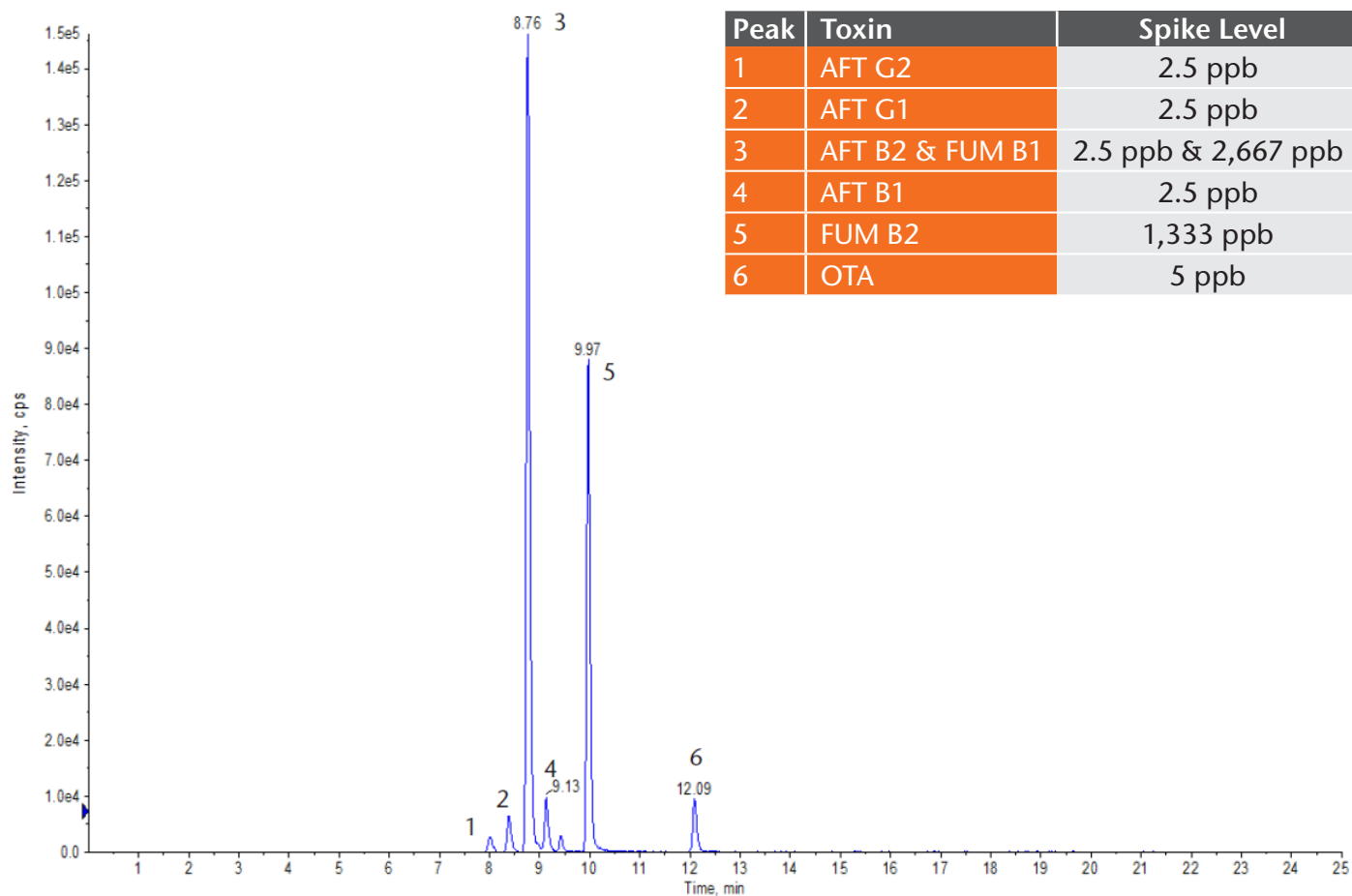
Mass Spectrometry Conditions

Instrument	Applied Biosystems API3000 LC-MS/MS with TurbolonSpray®
Mode	Multiple Reaction Monitoring (MRM) Mode with positive polarity
TurboProbe Temperature	450 °C
TurboProbe Heater Gas (Gas 2)	8 L/min (set manually)
IonSpray Voltage	+3,500 V
IonSpray Nebuliser Gas (Gas 1)	12
Curtain Gas	8
Collision Gas	12

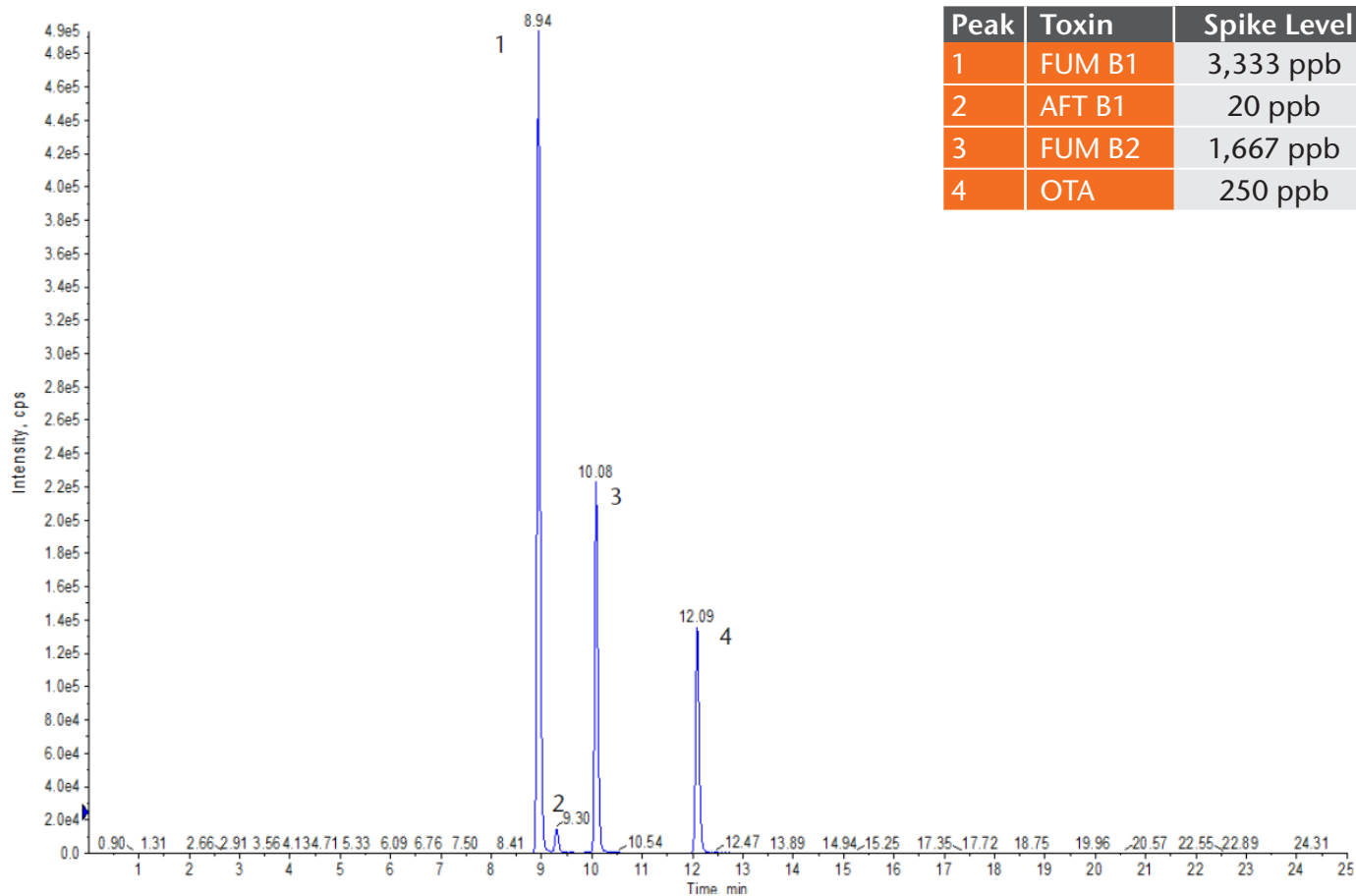
Instrument Setting

Toxin	Q1 Precursor Ion (m/z)	Q3 Product Ion (m/z)	Dwell Time (ms)		Focusing Potential (V)		Collision Exit Potential (V)
Aflatoxin G2	331.1 [M+H] ⁺	245.1 (Quantifier)	50	58.0	263.0	42.1	16.1
		189.1 (Qualifier)	50	58.0	263.0	55.8	17.0
Aflatoxin G1	329.1 [M+H] ⁺	243.1 (Quantifier)	50	48.9	240.0	37.7	16.3
		283.2 (Qualifier)	50	48.9	240.0	35.6	7.4
Aflatoxin B2	315.0 [M+H] ⁺	287.1 (Quantifier)	50	51.2	286.0	36.0	7.8
		259.0 (Qualifier)	50	51.2	286.0	40.5	6.2
Aflatoxin B1	313.0 [M+H] ⁺	285.1 (Quantifier)	50	63.4	310.0	32.8	7.3
		241.1 (Qualifier)	50	63.4	310.0	51.3	16.0
Fumonisin B1	722.4 [M+H] ⁺	352.4 (Quantifier)	50	62.7	319.0	50.1	10.0
		334.4 (Qualifier)	50	62.7	319.0	55.7	9.5
Fumonisin B2	706.5 [M+H] ⁺	336.4 (Quantifier)	50	45.8	230.0	51.0	9.1
		318.4 (Qualifier)	50	45.8	230.0	55.1	9.0
Ochratoxin A	404.1 [M+H] ⁺	239.1 (Quantifier)	50	41.2	238.0	31.3	6.0
		357.9 (Qualifier)	50	41.2	238.0	20.1	8.9

Example LC-MS/MS Total Ion Count Chromatogram for Maize



Example LC-MS/MS Total Ion Count Chromatogram for Animal Feed



Peak	Toxin	Spike Level
1	FUM B1	3,333 ppb
2	AFT B1	20 ppb
3	FUM B2	1,667 ppb
4	OTA	250 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

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