R-Biopharm Rhône Ltd

KOBRA® CELL
Instruction Manual

K01/V14/161110

R·BIOPHARM
RHÔNE LTD

Contents

1 Introduction to KOBRA® CELL

1.1 Intended Use

Confirmation of the presence of aflatoxins in a sample by HPLC requires derivatisation of aflatoxins B1 and G1 in order to enhance their natural fluorescence and make them more easily detected. Previously, the only options available for derivatising aflatoxins involved the use of trifluoroacetic acid (TFA), pyridinium bromide perbromide (PBPB) or iodine. All of these methods have significant limitations which can be overcome by use of the KOBRA® CELL.

Pre-column derivatisation with TFA requires the solution containing aflatoxin to be blown to dryness under a stream of nitrogen, potentially leading to a loss of toxin. Further limitations are that the reaction takes 30 minutes at 50 °C, and the TFA reagent is itself corrosive and harmful to handle.

The post-column PBPB method involves addition of the diluted reagent into the eluate from the HPLC column. The limitations of this method are the use of a second pump and the difficulty in dissolving the PBPB as well as the hazardous nature of the reagent.

Post-column derivatisation with iodine also has some limitations including the need for a second pump, water bath or oven which can be expensive. It is necessary to clean the equipment regularly in order to avoid iodine crystals forming inside the reaction coil. Finally, the iodine must be prepared fresh each day due to its unstable nature.

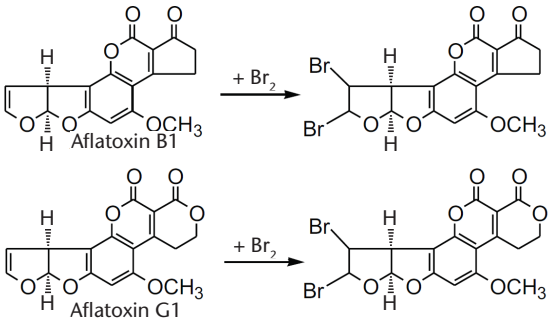
The KOBRA® CELL overcomes the problems relating to alternative derivatisation procedures. It is an electrochemical cell connected to an HLPC system downstream from the HPLC column and in line with the column effluent and the fluorescence detector. The KOBRA® CELL generates a reactive form of bromine for derivatisation of aflatoxins B1 and G1, resulting in enhanced fluorescence and thus more sensitive detection.

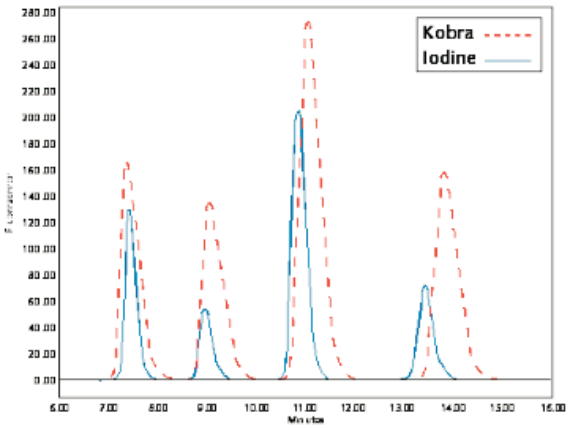
The KOBRA® CELL is used by hundreds of labs around the world and is mentioned in several EU and other international standard methods.

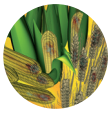
Introduction to KOBRA® CELL

1.2 Derivatisation Reaction

The aflatoxins and the mobile phase enter the KOBRA® CELL and the electrochemical reaction occurs generating the reactive form of bromine. The reaction between the reactive bromine and the aflatoxins must take place before the derivatised aflatoxins enter the fluorescence detector. Hence the length of the reaction coil is critical. A minimum reaction time of 4 seconds is required.







2 KOBRA® CELL Contents

2.1 Contents

- 1 x KOBRA® CELL
- 1 x Variable control current source
- 2 x Electrode connection leads
- 1 x Two pronged electrical adapter
- 1 x 50 cm length of 0.5 mm ID tubing
- 1 x Spare membrane

2.2 Reagents Required

- Distilled water
- Methanol
- Potassium bromide
- Nitric acid

2.3 Accessory Products

- 1 x KOBRA® CELL Installation Pack (Product Code: K03)*
- Guard column: Spherisorb ODS2, 5 µm, 5 cm x 4.6 mm or equivalent

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

2.4 Hazard

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.

2.5 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.

3 Assembly of the KOBRA® CELL

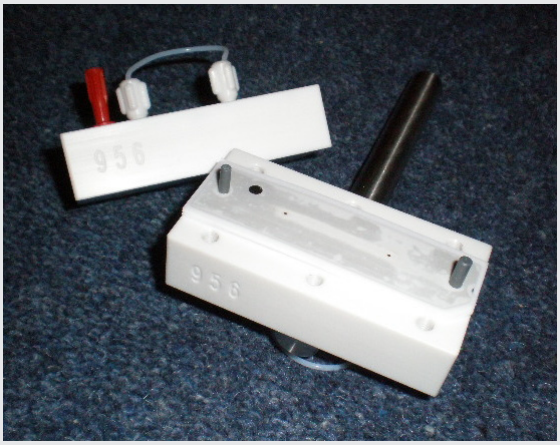
3.1 Components

6 locating screws
Plastic housing with red terminal (top)
Storage plugs & capillary tubing (for when KOBRA® CELL is not in use)
Teflon spacer: 0.1 mm (K09)
Platinum electrode (K05)
Teflon spacer: 0.25 mm (K07)
Ion exchange membrane (K02)
Teflon spacer: 0.25 mm (K07)
Spacer grid (K10)
Teflon spacer: 0.25 mm (K07)
Stainless steel electrode (K04)
Teflon spacer: 0.1 mm (K08)
Plastic housing with black terminal (bottom)

Assembly of the KOBRA® CELL

3.2 Assembly

Topside view of all KOBRA® CELL electrodes, membranes and spacers.



Smallest fitting hole for location on left side.

Largest fitting hole for location peg on right side.

Corner 'cut out' on bottom right.

4 KOBRA® CELL Installation

4.1 Precautions

- This device has only to be used with the supplied power pack.
- Never flush 100 % acetonitrile through the KOBRA® CELL as this can damage the membrane.
- Always ensure there is flow through the KOBRA® CELL before switching on the current source.
- Always turn off the KOBRA® CELL power source before turning off the HPLC pump.
- Always use HPLC grade solvents (with minimal benzene content) from a quality supplier and maintain fresh supplies of potassium bromide.
- Never use metal nuts or ferrules directly with the KOBRA® CELL.
- Do not over tighten locating screws as this will damage the threads.

4.2 Power Pack Notes

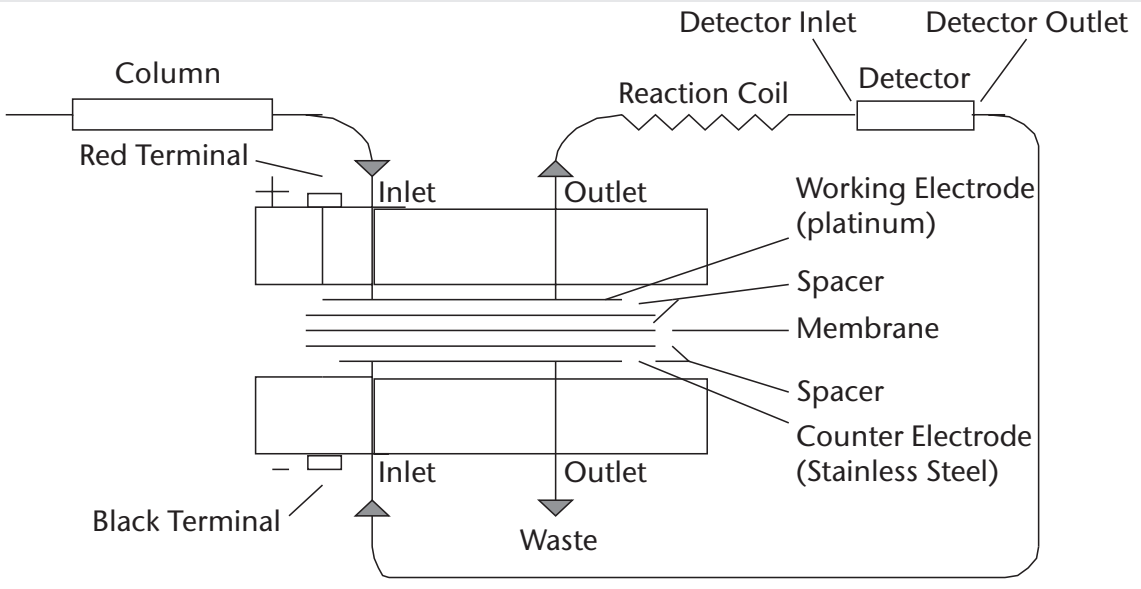
It should be noted that the KOBRA® CELL is now supplied with a 9 V power pack. The new 9 V power pack and adapter replaces the previous 15 V power pack and adapter. It is important to use the 9 V power pack in conjunction with the 9 V adapter. All new 9 V power packs and adapters will be labelled as such. Anything which is unmarked should be considered to be 15 V. Please do not interchange old and new adapters and power packs since this may have a detrimental effect on the KOBRA® CELL.

4.3 Power Pack Technical Specification

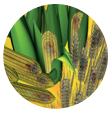
Power Supply	Primary 110 - 240 V Secondary 9 V
Exit Power Current Source	10, 20, 50, 100, 200 or 500 µA, approximately 2 %.
Maximum Output Power	9 V
Loading Time NICAD 9 V Accuracy	Approximately 1 hour 15 minutes.
Working Time with Battery / Accuracy	20 hours

KOBRA® CELL Installation

4.4 Installation Guide



Membrane:	Reichelt Thomapor Anion.
Capillary connections:	Valco 1/16 inch (do not use metal nuts or ferrules because these can damage the electrochemical properties of the cell).
Working pressure:	Max 2 bar.



KOBRA® CELL Installation

4.5 Installation Instructions

Caution: Do not over tighten. Cell connections should be made with plastic ferrules, not metal.

1. Unpack the KOBRA® CELL and check that all of the components are present.
2. Add 119 mg of potassium bromide and 350 µl of 4 M nitric acid to 1 l of mobile phase.
3. Immerse the tubing from the HPLC pump into the mobile phase.
4. Disconnect the storage plugs and capillary tubing from the KOBRA® CELL. Connect the HPLC column discharge to the KOBRA® CELL inlet using plastic ferrules (hand tightened) and a length of peek tubing supplied with the kit.
5. Connect the KOBRA® CELL outlet using ferrules (hand tightened) and a length of peek tubing to the detector inlet according to the table below.

   Caution: Use a guillotine tubing cutter to prevent distortion of the diameter of the tubing.
6. Connect the detector outlet to the KOBRA® CELL inlet using plastic ferrules (hand tightened) and peek tubing.
7. Connect the KOBRA® CELL outlet to waste using plastic ferrules (hand tightened) and peek tubing.
8. Switch on the HPLC pump and flush mobile phase through the system for approximately 5 minutes.
9. Connect the KOBRA® CELL power source to the cell, red lead to red terminal and black lead to black terminal. Set the power source to 100 µA and switch on.

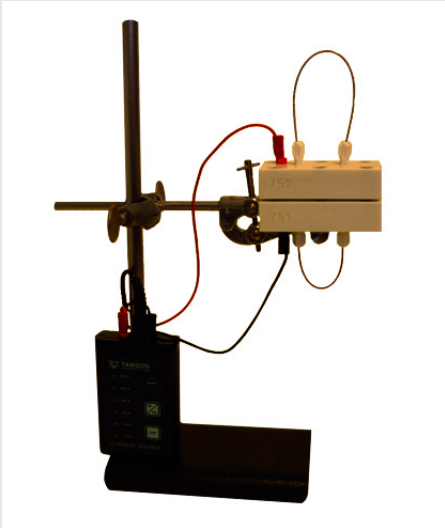
   Caution: Do not switch on without mobile phase flowing through the KOBRA® CELL or the membrane will be damaged.
10. After the HPLC has been allowed to stabilise (approx. 15 minutes) the KOBRA® CELL is ready to use.

Determination of Reaction Coil / Peek Tubing Length							
Flow Rate		0.5 ml/min	0.6 ml/min	0.7 ml/min	0.8 ml/min	0.9 ml/min	1.0 ml/min
Internal diameter of reaction coil / peek tubing (id)	0.2 mm	106.1 cm	127.3 cm	148.5 cm	169.8 cm	191.0 cm	212.2 cm
	0.25 mm	67.9 cm	81.5 cm	95.1 cm	108.6 cm	122.2 cm	135.8 cm
	0.4 mm	26.5 cm	31.8 cm	37.1 cm	42.4 cm	47.7 cm	53.1 cm
	0.5 mm	17.0 cm	20.4 cm	23.8 cm	27.2 cm	30.6 cm	34.0 cm
	0.6 mm	11.8 cm	14.1 cm	16.5 cm	18.9 cm	21.2 cm	23.6 cm
	0.8 mm	6.6 cm	8.0 cm	9.3 cm	10.6 cm	11.9 cm	13.3 cm
	1.0 mm	4.2 cm	5.1 cm	5.9 cm	6.8 cm	7.6 cm	8.5 cm

KOBRA® CELL Installation

4.6 Operating the KOBRA® CELL

- The KOBRA® CELL power pack is turned on by pressing the on/off button. To shut down the device you must hold the on/off button until all LEDs are turned off.
- You can choose the desired current (100 µA) by pressing the STEP key. If you hold the STEP key for longer than 3 seconds the key will repeat.



4.7 Error LED

- The LED will light up if there is no KOBRA® CELL attached to the current source.
- The LED will also light up when the KOBRA® CELL is dry and the flow of the current source is inhibited.

5

HPLC Conditions

HPLC Conditions	
Derivatisation	KOBRA® CELL at 100 µA setting
Guard Cartridge	Inertsil ODS-3 5 µm, 4 mm x 10 mm or (Hichrom) equivalent
Analytical Column	Inertsil ODS-3V 5 µm, 4.6 mm x 150 mm (Hichrom) or equivalent
Mobile Phase	Water : Methanol (60 : 40 v/v) Add 119 mg of potassium bromide and 350 µl 4 M Nitric Acid to 1 litre of mobile phase
HPLC Pump	To deliver mobile phase
Flow Rate	1.0 ml/minute
Fluorescence Detector	Excitation: 362 nm
	Emission: 425 nm (B1 and B2) 455 nm (G1 and G2)
Column Heater	Maintain guard and analytical columns at 40 °C
Integrator / Data Control System	From preferred supplier
Injector	Autosampler / Reodyne valve
Injection Volume	100 µl
Elution Order	G2, G1, B2, B1.

6 KOBRA® CELL Maintenance

6.1 Storage

Always store the KOBRA® CELL filled with water in order to keep the membrane wet.

6.2 Daily Cleaning

The HPLC system can be left running overnight at a reduced flow rate (e.g. 0.1 ml/min) and with the fluorescence detector and KOBRA® CELL power source turned off, if necessary. Alternatively, a better practice to prolong the life of the system is to clean each day, as follows:

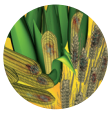
1. Turn off KOBRA® CELL power source and fluorescence detector.
2. Switch off HPLC pump.
3. Disconnect the KOBRA® CELL from the HPLC system and reconnect the HPLC column directly to the fluorescence detector.
4. Change the mobile phase to 100 % acetonitrile and put the HPLC column discharge to waste.
5. Switch on the HPLC pump and flush the system for at least 30 minutes before switching off the system.
6. Manually flush the KOBRA® CELL through with distilled water several times using a syringe.
7. Store the KOBRA® CELL overnight with water inside the cell by closing off both ports on each housing using the storage plugs and capillary tubing supplied with the cell.
8. The next day, change the mobile phase from 100 % acetonitrile back to the normal mobile phase of potassium bromide and nitric acid, and flush the HPLC system through for at least 30 minutes before re-connecting the KOBRA® CELL.

   Caution: 100 % acetonitrile will damage the KOBRA® CELL membrane.

6.3 Monitoring Performance

It is necessary to regularly monitor the performance of the KOBRA® CELL in order to detect any deterioration in the membrane contained within. The performance should be checked at the time of installation and then weekly by comparing the peak areas of a known aflatoxin standard. The same should also be done in order to monitor the deterioration of the lamp in the detector.

Deterioration of the membrane will occur over a period of time depending upon the frequency of use and the type of samples analysed. When the performance becomes unacceptable the ion-exchange membrane should be replaced. Normally, it is found that even under extreme workloads the membrane need not be replaced for at least 6 months or 1,000 injections.



KOBRA® CELL Maintenance

6.4 Changing the Membrane

Change the membrane when a decrease in performance is observed which cannot be attributed to the detector lamp. Additional membranes are available separately (K02).

1. Using a screw driver take off the rigid plastic housing of the KOBRA® CELL by removing the 6 locating screws.

2. Carefully separate the top housing, and remove in turn the internal layers of the cell using tweezers. Make a note of the position and orientation of each layer as it is removed.

3. Continue removing the internal layers until the membrane is exposed. Before removal inspect the membrane for damage, discolouration and orientation. During the life of the KOBRA® CELL the bromination reaction causes the centre of the membrane to lose it's colour. No holes should be visible in the membrane other than the pre-cut holes for the locating pins at either end of the membrane. Note the orientation of the membrane.

4. Remove the membrane from the KOBRA® CELL ready for replacement using tweezers.

5. Carefully remove the spare membrane, holding it by one end with the tweezers rather than the middle.

6. Position the replacement membrane over the different sized locating pins, according to the size of pre-cut holes in the membrane.

   Caution: Do not allow the membrane to dry out. Add distilled water if necessary.

7. Carefully replace the layers to be positioned on top of the membrane, remembering their correct order and orientation.

8. Secure the layers using the 6 locating screws.

7 General Information

7.1 Quality

RBR products are developed, manufactured, tested and dispatched under an ISO 9001 and ISO 13485 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.

7.2 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.

7.3 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.

R-BIOPHARM RHÔNE LTD

R-Biopharm Rhône Ltd
Block 10 Todd Campus
West of Scotland Science Park
Acre Road, Glasgow G20 0XA
Phone: +44 (0) 141 945 2924
Fax: +44 (0) 141 945 2925
www.r-biopharm.com

R-BIOPHARM
RHÔNE LTD