Instructions for use

FARLIB® LIBIOS CELL

Reference : **KB-CELL**

Electrochemical derivatisation cell for HPLC post-column online bromine derivatisation of Aflatoxins prior to fluorescence detection

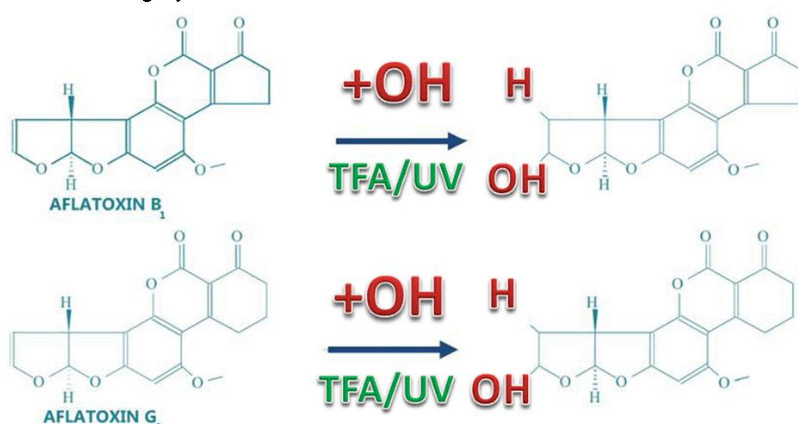
Dynamic Test Kits
for R&D
and Quality Control

www.libios.fr

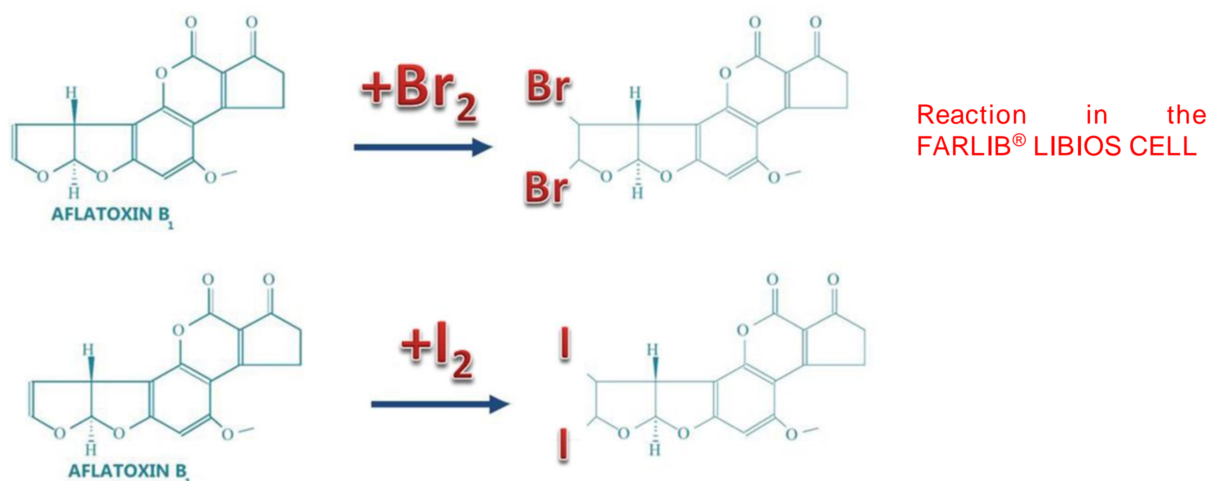
Introduction:

High performance liquid chromatographic method, with post column derivatisation after immunoaffinity column clean up and fluorescence detection, is the worldwide reference method for accurate aflatoxin B1, B2, G1 and G2 determination in varieties of Food and Feed products.
Aflatoxin B1 and G1 have very weak fluorescence properties. To overcome it requires pre- or post-column derivatisation processes to improve their detection limits. This should be done to comply with low maximum limit concentrations in food and feed required by different regulations worldwide.

As it was mentioned, derivatisation of aflatoxins are classified to 2 groups pre- or post-column derivatisation processes. However the root reaction is very similar.
In these reactions aflatoxins B1 and G1 are transformed to the either Hydroxylated or Halogenated derivatives aflatoxins B2a and G2a respectively.
Hydroxylated derivatives through addition of a hydroxyl (-OH) functional group attached to the second furan ring of aflatoxin B1 and G1 could be produced using either hydrolyzed by trifluoroacetic acid (TFA) at 50°C for 30 minutes as a pre-column derivatisation processes or using photochemical derivatisation under UV light radiation at 250 nm for a few seconds as a post-column derivatisation processes. The results are conversion into a highly fluorescent aflatoxin derivatives.



Halogenated derivatives through addition of a bromine (-Br) or Iodine (-I) functional group attached to the second furan ring of aflatoxin B1 and G1 could be produced using either bromine and iodine as reagent.
The formation of highly fluorescent aflatoxin B1 and G1 derivatives of these halogens, shall make the detection possible at very low levels that required.
Brominated derivatives of aflatoxin B1 and G1 could be produced using either electrochemically or using bromine reagent pyridinium hydrobromide perbromide (PBPB) through an auxiliary pump.



Reaction in the FARLIB® LIBIOS CELL

Cell innovations of FARLIB® LIBIOS CELL:

- FARLIB® LIBIOS CELL body has excellent physical and chemical resistance compare to other cells available on the market that using other materials such as teflon, thanks to PEEK's (Polyether ether ketone) superior strength-to-weight ratio as well as its excellent resistance to chemicals, abrasion and hydrolysis and other properties.
- The second advantages is that its current source benefit from connecting to a 5V USB charger port of a PCs, laptops, and other hardware, reducing one connection to the main.
- 2 red light led for error.
- Engraved cell to avoid manipulation error.
- Improved separation grid to supress the pressure increase effect and prevent breakage of the flow cell detector.

Advantages:

- high increase of aflatoxins fluorescence activity
- high reproducibility of results
- derivatisation in 4 seconds at ambient temperature, no heating block necessary
- no daily preparation of derivatising reagent necessary
- no stability problems of mobile phase even after addition of potassium bromide and nitric acid
- no handling with halogeneous solutions (for example iodine)
- no additional pump for addition of derivatising reagent
- no corrosion of pumps
- low maintenance costs
- simple to install/uninstall

FARLIB® LBIOS CELL components:

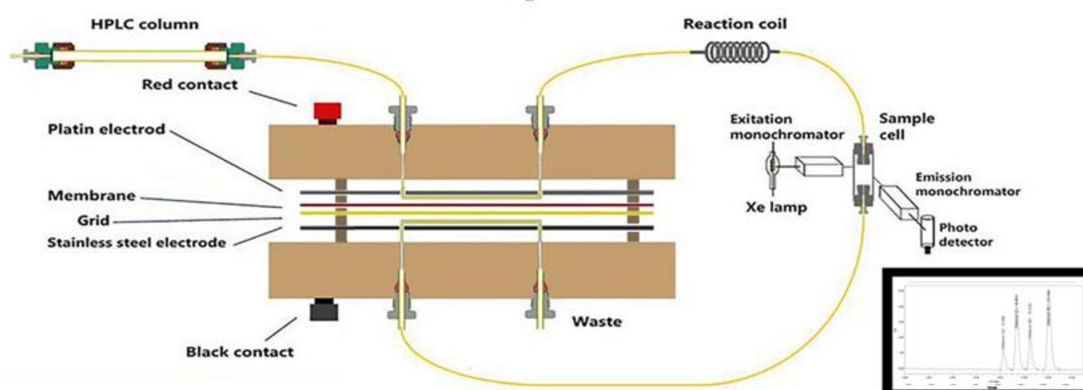
1 x FARLIB® LIBIOS CELL (PEEK COMPLETE BODY)
1 x Current Source + Mini-USB Connection Cable
2 x Electrode Connection Cables
1 x Spare Membrane

Important notes:

Hazard: Aflatoxins are very hazardous substances. Suitable protective clothing, including rubber gloves, safety glasses and laboratory coats should be worn throughout the assay.

- Ensure that the working pressure when the FARLIB® LIBIOS CELL is connected doesn't exceed 2 bars at the flow cell detector.
- When not using the FARLIB® LIBIOS CELL always store it filled with water in order to keep the membrane wet.
- Never flush 100% v/v organic solvents through the FARLIB® LIBIOS CELL as this can damage the membrane.
- Always ensure there is flow through the FARLIB® LIBIOS CELL before plugging in the current source.
- Unplug the FARLIB® LIBIOS CELL current source before turning off the HPLC pump. Always use HPLC grade solvents from a quality supplier, and maintain fresh supplies of potassium bromide.
- Never connect metal nuts or metal tubes directly with the FARLIB® LIBIOS CELL. These can damage the cell.
- Always use plastic tubing's (teflon, peek...) for connections. Always keep tube internal diameter's the same.

Installation of the FARLIB® LIBIOS CELL



a) Prepare 1 litre of mobile phase, MeCN:MeOH:H2O (200:300:600 v/v) containing 120 mg of potassium bromide (KBr) and 350 µL 4M nitric acid (HNO_3). All solvents should be HPLC-grade.
b) Disconnect all capillary tubing from the FARLIB® LIBIOS CELL and connect the HPLC column with a plastic tubing to the FARLIB® LIBIOS CELL inlet (marked "COLUMN"). Connect FARLIB® LIBIOS CELL outlet (marked "FLD-in") with a plastic tubing to the detector inlet - this length of tubing is critical, and the length is dependent upon flow rate and internal diameter (I.D.) of the tubing. Length calculation see equation and table 1.

Table 1 — Equation: 8.5 * Flowrate [ml/min] / I.D.² [mm]

	0.4ml/min	0.5ml/min	0.6ml/min	0.8ml/min	1.0ml/min
0.20mm I.D.	84.9 cm	106.1 cm	127.3 cm	169.8 cm	212.3 cm
0.25mm I.D.	54.3 cm	67.9 cm	81.5 cm	108.6 cm	135.8 cm
0.40mm I.D.	21.2 cm	26.5 cm	31.8 cm	42.4 cm	53.1 cm
0.50mm I.D.	13.6 cm	17.0 cm	20.4 cm	27.2 cm	34.0 cm
0.80mm I.D.	5.3 cm	6.6 cm	8.0 cm	10.6 cm	13.3 cm

c) Connect the detector outlet with a plastic tubing to the FARLIB® LIBIOS CELL inlet (marked "FLD-out") and the FARLIB® LIBIOS CELL outlet (marked "WASTE") with a plastic tubing to waste. All connections are made to hand tightness (CAUTION : do not over tighten) and should be plastic not metal or stainless steel.
d) Switch on HPLC pump.
e) Connect the FARLIB® LIBIOS CELL current source to the cell, red lead to red terminal and black lead to black terminal. Plug in current source on the PC USB port and switch it on. One green and 1 orange LED lights on the current source indicate the function as listed in the table:

LED		Meaning	Repair
OK	Error 1		
on	off	correct function	--
on	on	Cell not connected No additives in mobile phase	Verify connection of red lead to red terminal and black lead to black terminal Add KBr and HNO_3 (see HPLC conditions, mobile phase)

f) After the HPLC has been allowed to stabilise (no more baseline drift, 20-30 minutes) the FARLIB® LIBIOS CELL is ready to be used.

HPLC Conditions (Recommendation)

Guard column	Optimal guard column ODS-H 1 3 µm - 4.6 mm x 20 mm or equivalent
Analytical column	InertSustain® AQ C18 analytical column ODS1 5 µm - 4.6 mm x 250 mm or equivalent
Fluorescence detector	365 nm excitation 435 nm emission
Mobile Phase	Isocratic:MeCN:MeOH:H2O 200:300:600 To one litre of mobile phase add 120mg of potassium bromide and 350µL 4M nitric acid. All solvents should be HPLC-grade
Flow rate	1 ml/min
FARLIB Current Source	100 µA
Injection volume	100 µl
Elution sequence	Aflatoxin G_2, G_1, B_2, B_1

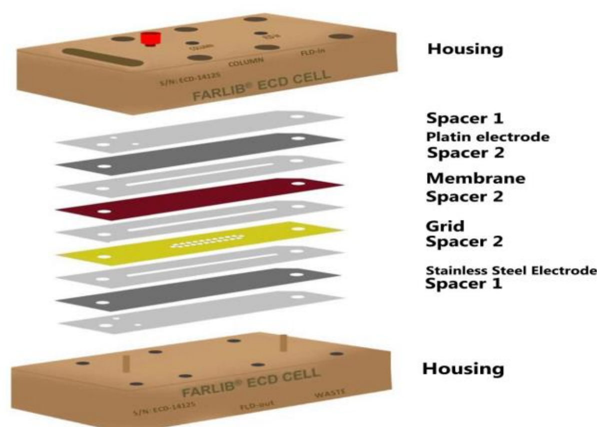
Daily cleaning routine for the FARLIB® LIBIOS CELL

The HPLC system can be left running overnight at a reduced flow rate (for example 0.1ml/min) and with the fluorescence detector and FARLIB® LIBIOS CELL current source unplugged, if necessary. Alternatively, a better practice to prolong the life of the system is to clean each day, as follows:

1. Switch off FARLIB® LIBIOS CELL current source and fluorescence detector.
2. Switch off HPLC pump.
3. Disconnect FARLIB® LIBIOS CELL from HPLC system, and reconnect HPLC column directly to fluorescence detector.
4. For washing the HPLC column use a mobile phase of acetonitrile (100% v/v).
5. Before turning off the system, flush it for at least 30 min.
6. Meanwhile manually flush FARLIB® LIBIOS CELL through with 5-10 ml distilled water using a syringe. Then store FARLIB® LIBIOS CELL with water inside the cell by closing off both ports on each housing with a tube bridge. This avoids a drying out of the membrane during longer storage periods.

Monitoring the sensitivity of the FARLIB® LIBIOS CELL

It is necessary to regularly monitor the performance of the FARLIB® LIBIOS CELL in order to detect any deterioration in the membrane contained within. The sensitivity should be checked at the time of installation and then weekly by comparing the peak areas of a known aflatoxins standard solution (for example, by using our product STD-AFBG-250-4 (4mL) ; certified standard solution containing a mixture of aflatoxins B_1, B_2, G_1, G_2 at 250ng/ml and per toxin) from one week to another week.



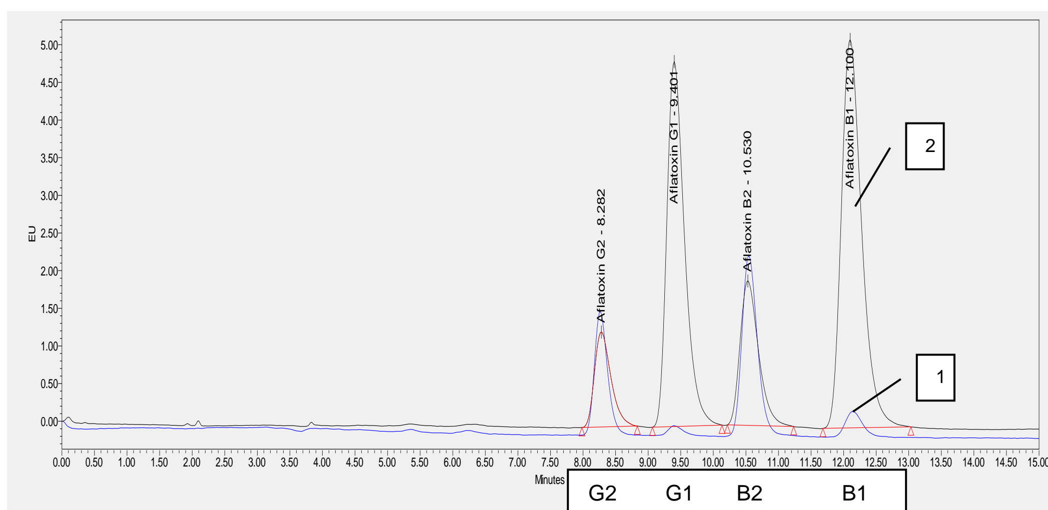
Depending upon the frequency of use and the type of samples analysed, deterioration will occur over a period. When the level of sensitivity becomes unacceptable the membrane should be replaced.

Normally, it is found that even under extreme workloads the membrane need not be replaced for at least 6 months.

Maintenance: Changing the membrane:

- Change the membrane when a decrease in sensitivity of aflatoxins B_1 and G_1 is observed, and the sensitivity of the other 2 aflatoxins stays the same. Additional membranes are available separately (contact LIBIOS).
- Using the hexagon key crosswise, remove the six locating screws in the top housing over cross.
- Carefully separate the top housing, and remove in turn the internal layers of the cell using forceps. Make a note of the position and orientation of each layer as it is removed. Continue removing the internal layers until the brown membrane is exposed.
- Remove the old membrane and replace it by a new membrane just having taken it out of the foil pouch. Take care not to touch the middle of the membrane with hands or fingers. DO NOT ALLOW THE MEMBRANE TO DRY-OUT. ADD DISTILLED WATER IF NECESSARY.
- Carefully replace the layers to be positioned on top of the membrane remembering their correct order and orientation. Secure the layers in by re-fitting the six locating screws in the housing over cross. Tighten the screws just by hand, do not over tighten the screws. Maximum 2 Nm.
- Using a syringe, pass distilled water through the “COLUMN” port and make sure that the water comes out from the “FLD-in” side without needing to apply any pressure.
- Using a syringe, pass distilled water through the “FLD-out” port and make sure that the water comes out from the “WASTE” side without needing to apply any pressure.
- After changing the membrane, ensure that the working pressure when the FARLIB® LIBIOS CELL is connected doesn’t exceed 2 bars at the flow cell detector.
- Regularly monitor the sensitivity of the FARLIB® LIBIOS CELL in order to detect any significant deterioration of the membrane contained within.

Chromatogram without Derivatisation (“1”) versus FARLIB® LIBIOS CELL derivatised Aflatoxins (“2”)



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