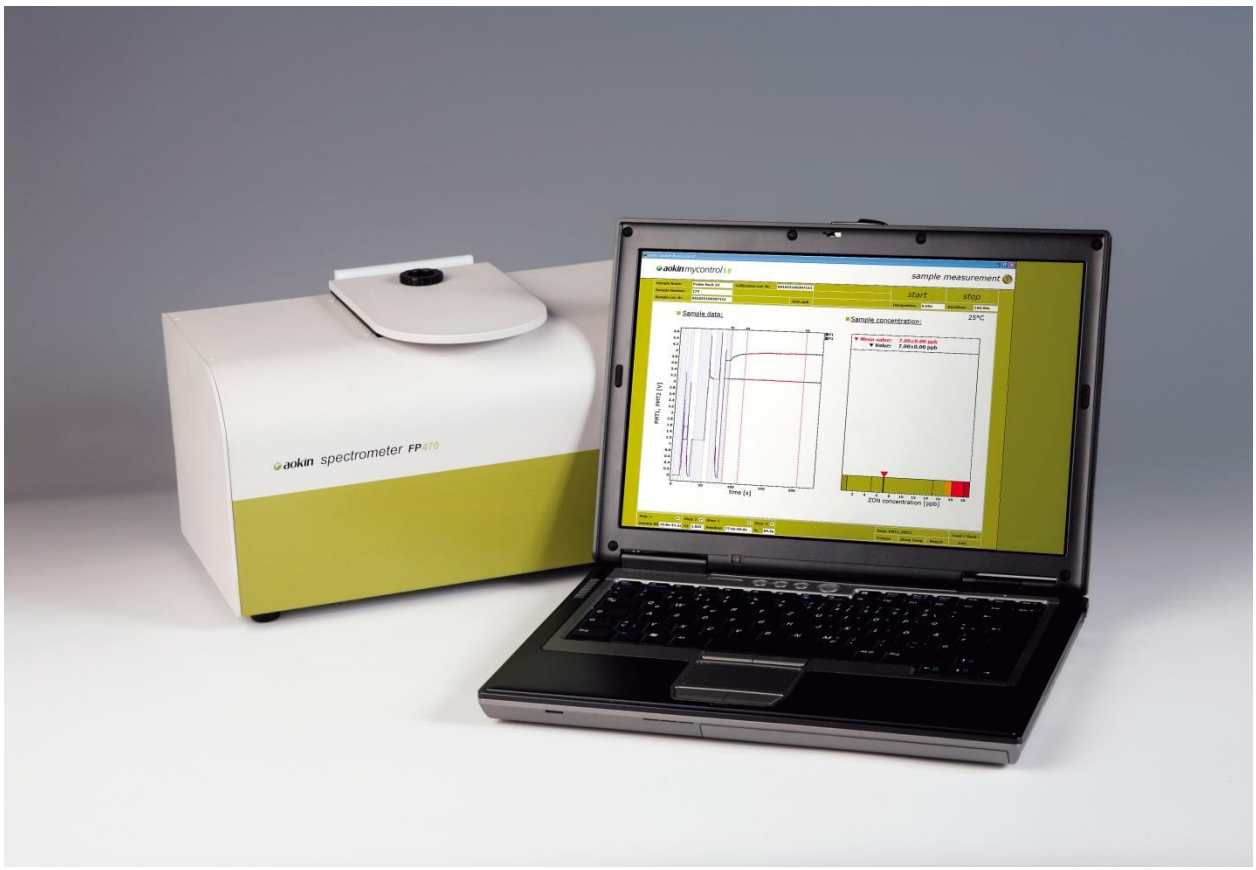
INSTRUCTIONS FOR USE

aokin mycontrol AFLA SPE

Order No.: MY-QC-03

Sample preparation with *aokin*QuickClean columns (SPE) – for corn and wheat samples



*aokin*mycontrol*AFLA SPE*

Analytical-kit for rapid and quantitative determination of Aflatoxin total in **corn and wheat.**

Materials:

aokin*mycontrol****AFLA SPE*** (Order No.: MY-QC-03-100)

Package content
Additional material may be needed (Methanol HPLC grade)

A) *Materials for sample preparation:*
aokin*ExtractionSolvent* ***AFLA*** / or ***aokin****Extraction-Solvent Concentrate* ***AFLA*** **(in this case please add Methanol HPLC grade)**
aokin/C*Dilute 10x*
aokin*ExtractionSalt* ***AFLA*** + spoon
aokin*QuickClean* ***AFLA,*** centrifuge columns
Filter paper, Reaction tubes 2 mL

Figure 1: ***aokin****QuickClean column with reaction tube and Extraction solvent (1 L bottle)*

B) *Materials for analytical measurement:*
aokin*ReactionBuffer,* Reaction buffer
aokin*mycontrol****OTA/AFLA SPE,*** Additive (black cap), ADD-OTA/AFLA SPE, (for 5 analyses each)
aokin*mycontrol****AFLA,*** Reagent 1 (white cap), F-AFLA, (for 5 analyses each)
aokin*mycontrol****AFLA,*** Reagent 2 (green cap), A-AFLA, (for 5 analyses each)

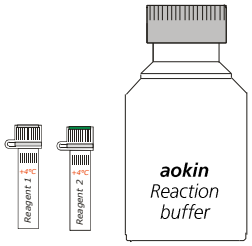


Figure 2:
Reagent 1 (white cap),
Reagent 2 (green cap),
Reaction buffer (1 L bottle)

C) *Materials for internal quality control:*
aokin*mycontrol* ***AFLA,*** negative control AFLA (transparent), for zero value measurements
aokin*mycontrol****AFLA,*** Reagent 1 (white cap), F-AFLA, (for 5 analyses each)
aokin*mycontrol****AFLA,*** Reagent 2 (green cap), A-AFLA, (for 5 analyses each)

Note: All substances provided are precisely weighed and calibrated. Control of the volume and concentration of the individual solutions are essential for the precision of the analysis.
Precaution: The extraction solvent may contain methanol. Work with professional care.
Storage Conditions: Reagents 1 and 2 must be stored at temperature of +4°C. All other components may be stored at room temperature.
Quality Control: All materials and reagents are prepared according to strict quality control protocols. Exchanging reagents between kits having different Lot-numbers will lead to erroneous results and is not permitted.

Order Information:
aokin*mycontrol****AFLA SPE*** (Order No.: MY-QC-03-100)

Introduction

aokin*mycontrol****AFLA SPE*** is a rapid and precise quantitative method for the analysis of Aflatoxin B1 (AFLA). It includes a sample preparation with solid phase extraction (SPE) columns. Samples of corn and wheat can be analysed with a limit of detection of 1,5 µg/kg.
aokin*mycontrol****AFLA SPE*** is available with a calibration, which has been validated for wheat products and others like animal feed. Please use professional care and check the accuracy by regularly analyzing reference materials (e.g. ***aokin****ReferenceMatrix-Materials*) or standards. Participation in proficiency tests is recommended.
aokin will gladly assist you customising the test for your specific sample type and application. Please do not hesitate to contact us.

Sample	wheat, corn
Time required for sample preparation	4 minutes
Time required for measurement	3 minutes
Analysis	
	Measurement range [µg/kg]
Range 1	0,6 – 16,0
Range 2	1,2 – 32,0
Range 3	2,7 – 72,0

Aflatoxin

Aflatoxins are mycotoxins produced by molds such as *Aspergillus flavus* and *Aspergillus parasiticus.* Aflatoxin B1 is acutely toxic and among the most carcinogenic substances known. As a consequence, it is necessary to monitor AFLA content in food and feed products.

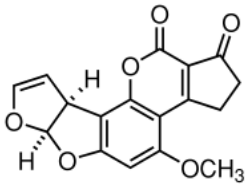


Figure 3: Chemical formula for Aflatoxin B1,
$C_{17}H_{12}O_6$Molecular weight: 312,3 g/mol

Recommended Accessories

All required materials are available from ***aokin***.
Tel.: +49 30 9489 2160

	Order No.
aokin*extractor* (food blender)	EX-07-06
aokin*watchbox* (timer for food blender)	EX-07-06-4
Weighing scale, d = 0,01 g	LB-03-04
Eppendorf centrifuge, variable g-force	LB-04-04
Variable pipette (1000 µl)	LB-04-05-1000
Pipette tips (1000 µl)	LB-04-08-1000
Funnel	LB-05-04
Dispensette	LB-08-01
aokin*ReferenceMatrixMaterial*	RMM-03

Sample preparation

The following protocol is an example. The quantification ranges are dependent on dilutions. Actual volume settings in the software may vary.

Note: It is of critical importance to use the correct sample preparation protocol for each determination. Use volumes displayed in the ***aokin*** software.

1. Quality control

Included in the analytical kit there are following additional materials for your internal quality control: ***Reagent 1***, ***Reagent 2***, ***Additive*** and negative control samples (labelled ***negative control***, corresponding to samples free of mycotoxin) and a positive control sample.
Please perform measurements of negative controls regularly, this ensures the accuracy of your determinations.
If you notice increased values, change cuvette and repeat measurement. If sample results remain high, contact the ***aokin*** team.

2. Sample collection, grinding and mixing

The analysis sample is collected, ground, and mixed (homogenised) according to an approved procedure. Small sample volumes may be ground and mixed using the ***aokin****extractor*.

3. Weighing and extraction

Weigh 15 g of your sample, add one spoon (1,5 g) of ***aokin****ExtractionSalt* ***AFLA*** and 30,4 g extraction solution (35 ml ***aokin****ExtractionSolvent* ***AFLA*** at 20°C) directly into the extraction beaker (Figure 4). Preferentially the exact volume is applied using a dispensette.

Figure 4: Weighing

Close the extraction beaker with the lid (with the blending knifes). Blend for 3.5 minutes. The recommended protocol has blending times alternating with resting time to avoid heating of the sample and is as follows: mix for 30 seconds, pause for 1 minute, mix for 30 seconds and so on (until 3.5 minutes of blending time).
Use the ***aokin****watchbox* (a preprogrammed timer) to conveniently and automatically complete this extraction protocol.

Figure 5: Extracting with the ***aokin****extractor (blender)*

3. Filtration:

Place the filter on a suitable funnel and the funnel onto a collection container. Open the extraction beaker and pour the contents over the filter and collect the filtrate. Discard the filter paper and filter cake. Shake/stir the filtrate to ensure homogeneity.

Figure 6: Filtration

4. Use of *aokin*QuickClean column

Place first ***aokin****QuickClean AFLA* column in a collection tube and add 900 µl of the filtrate (Figure 7). Place it in the centrifuge and spin for 3 minutes at 5000 x g. Place second ***aokin****QuickClean AFLA* column in a collection tube and add ≥ 650 µL of the purified filtrate. Place it in the centrifuge and spin for 3 minutes at 5000 x g .

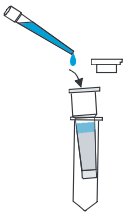


Figure 7: Pipetting of the extract onto the ***aokin****QuickClean****AFLA*** *column*

5. Dilution

Pipet 400 µL of the purified filtrate and 650 µL of deionized water in a reaction tube and mix intensely. This step is equal to a 1:2.6 dilution. Spin for 3 minutes > 16.000 x g and transfer the supernatant into a clean tube. Your sample is now ready for analysis.

6. Analyzing

Use the column filtrate for the analysis in the ***aokin****spectrometer****FP470***.

Please follow detailed instructions for spectrometer use (***aokin****spectrometer****FP470*** & ***aokin****LHW03* Instructions for use).

This includes:

1) Place ***Reagents 1*** and ***2*** into position A6 and B6, and place ***Additive*** into position B1 of the sample rack of your spectrometer.
2) Place a 25 ml ***Clean1-glass container, filled with Clean1 solution*** into the Clean1-position, on the left side, next to the palette.
3) Place a 25 ml ***neg. control AFLA SPE-glass container, filled with negative control AFLA SPE solution = 4 mL aokin****Extraction Solvent* ***AFLA*** + ***6,5 mL*** deionized water in the Clean2-position, on the left side of the palette.
4) Place an empty 2 mL vial in position A1 of the palette.
5) Place an empty waste bottle into the holder. Check presence of ***Reaction buffer*** and check if tubing is below the surface.
6) Place a new cuvette with a clean stirrer into the spectrometer.

Conversion factor: analyte concentration in cuvette (nM) to amount in sample (µg/kg)

aokinmycontrol AFLA SPE Standard

Step 1: Extraction

Sample mass: m_{Sample} = 15 g

Volume extraction solvent: $V_{Extraction\ solvent}$ = 35 mL

Molar mass Aflatoxin B1: $MW_{Afla} = 312{,}3\ \left[\frac{g}{mol}\right]$

Mycotoxin concentration in the sample extract:

$$c\left[\frac{\mu mol}{L}\right]_{Extract} = \frac{m_{Sample}[kg]}{V_{Solvent}[l] * MW_{Mykotoxin}\left[\frac{g}{mol}\right]} * c\left[\frac{\mu g}{kg}\right]_{Sample} = \frac{0.015}{0.035 * 312.3} * w\left[\frac{\mu g}{kg}\right]_{Sample} = 0.0013723 * c\left[\frac{\mu g}{kg}\right]_{Sample}$$

Step 2: Purification

with *aokin QC AFLA*

*- Volume sample extract load to the **aokin** QuickClean column:* $V_{loaded\ sample\ extract}$ = 0,9 ml

*- Volume "**aokin** Elute" used for the elution of the column:* V_{elute} = 0,9 ml

$$c\left[\frac{\mu mol}{l}\right]_{Eluate} = \frac{V_{load\ [ml]}}{V_{elute\ [ml]}} * c\left[\frac{\mu mol}{l}\right]_{Extract} = \frac{0.9}{0.9} * c\left[\frac{\mu mol}{l}\right]_{Extract} = 1 * c\left[\frac{\mu mol}{L}\right]_{Extract}$$

Step 3: Dilution

- Volume Eluate: V_{eluate} = 0,4 ml

- Total volume: V_{total} = 1,05 ml (0,65 ml $_{Water}$ + 0,4 ml $_{Eluate}$)

$$c\left[\frac{\mu mol}{L}\right]_{Eluate} = \frac{V_{eluate}[mL]}{V_{total}\ [mL\}} * c\left[\frac{\mu mol}{L}\right]_{Extract} = \frac{0{,}4}{1{,}05} * c\left[\frac{\mu mol}{L}\right]_{Extract} = 0.38095 * c\left[\frac{\mu mol}{L}\right]_{Extract}$$

Step 4: Measurement

aokin FP 470 / *LHW 03*

Sample volume: $V_{centrifuged\ dilution} = V_{Sample} = 900\ \mu l$

Total volume in the cuvette: $V_{Cuvette} = 2600\ \mu l$

Mycotoxin concentration in the cuvette:

$$c\left[\frac{\mu mol}{L}\right]_{Cuvette} = \frac{V_{Sample}\ [\mu L]}{V_{cuvette}\ [\mu L]} * c\left[\frac{\mu mol}{L}\right]_{Eluate} = \frac{900}{2700} * c\left[\frac{\mu mol}{L}\right]_{Eluate} = 0.333 * c\left[\frac{\mu mol}{L}\right]_{Eluate}$$

Conversion factor: Extraction, Purification and Measurement

It follows the conversion factor from 1 to 4 above:

$$c\left[\frac{\mu mol}{L}\right]_{Cuvette} = 0{,}0013723 * 1 * 0.38095 * 0.333 * c\left[\frac{\mu g}{kg}\right]_{Sample} = 0.0001742 * c\left[\frac{\mu g}{kg}\right]_{Sample}$$ or

$$c\left[\frac{\mu g}{kg}\right]_{Sample} = \frac{1}{0.1742} * c\left[\frac{nmol}{L}\right]_{Cuvette} = \mathbf{5.740} * c\left[\frac{\mathbf{nmol}}{\mathbf{L}}\right]_{Cuvette}$$

Dilution scheme of the standard solution sent for control purposes:
AFLA B1 [300 ng/mL] [960.61 nM] in Acetonitrile, 50 µl

Calibration:

Dilution scheme (example for standard experimental setup of 900 µl sample into 2700 µl of total cuvette volume):

AFLA B1 [300 ng/mL] [960.61 nM in vial]
↓ **1:80 diltution** in *negative control AFLA SPE*
3.75 ng/mL (12 nM in vial → **4.00 nM in cuvette**)
↓ **1:2 diltution** in *negative control AFLA SPE*
1.875 ng/mL (6 nM in vial → **2.00 nM in cuvette**)
↓ **1:2 diltution** in *negative control AFLA SPE*
0.9375 ng/mL (3 nM in vial → **1.00 nM in cuvette**)
↓ **1:2 diltution** in *negative control AFLA SPE*
0.4688 ng/mL (1.5 nM in vial → **0.50 nM in cuvette**)

negative control AFLA SPE
(→ **0.00 nM in cuvette**)

Positive control:

Dilution scheme (example for standard experimental setup of 900 µl sample into 2700 µl of total cuvette volume):

AFLA B1 [300 ng/mL] [960.61 nM in vial]
↓ **1:100 diltution** in *negative control AFLA SPE*

900 µl in RANGE 1
3.00 ng/mL (9.61 nM in vial ----------→ 3.20 nM in cuvette
in calculated volume for solid sample → 18.365 µg/kg)

450 µl in RANGE 2
3.00 ng/mL (9.61 nM in vial ----------→ 1.60 nM in cuvette
in calculated volume for solid sample → 9.183 µg/kg)

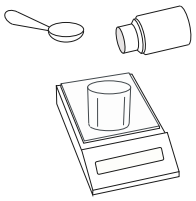
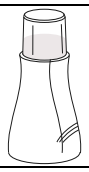
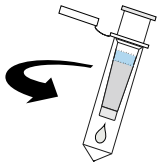
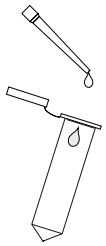
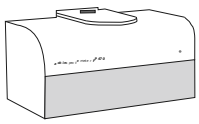
200 µl in RANGE 3
3.00 ng/mL (9.61 nM in vial ----------→ 0.71 nM in cuvette
in calculated volume for solid sample → 4.075 µg/kg)

Aflatoxin / standard samples:

- Recommended for <u>corn</u> flour samples

Procedure:

***aokin**mycontrol AFLA SPE Standard*

Extraction		**Weighing:** 15 g sample 1,5 g ***aokin**ExtractionSalt AFLA* 35 mL ***aokin**ExtractionSolvent AFLA*
		**Extraction:** 3,5 min mixing with ***aokin**watchbox*
		**Filtration:** collect filtrate (discard filter cake)
Purification		**SPE-Filtration:** 1. column: 900 µL filtrate on ***aokin**QuickClean* column 3 min centrifuge at 5000 x g 2. column: ≥ 650 µL filtrate of the 1st ***aokin**QuickClean* on 2nd ***aokin**QuickClean* column 3 min centrifuge at 5000 x g, use second column filtrate for measurement
Dilution		**Dilution:** 400 µL purified filtrate 650 µL deionized water 3 min centrifuge at > 16.000 x g transfer supernatant into clean 2 mL reaction tube
Measurement		**Automatic Analyse (FP470 / LHW03)** place the 2ml reaction tube in the sample holder of the *LHW03* 1400 µL ***aokin** Reaction buffer* 200 µL ***aokin**mycontrol AFLA SPE Additive* 900 µL sample *(diluted 1:1 - RANGE 1)* *(diluted 1:2 - RANGE 2)* *(diluted 1:4,5 - RANGE 3)* 100 µL ***aokin**mycontrol AFLA* Reagent 1 100 µL ***aokin**mycontrol AFLA* Reagent 2
	AFLA = Aflatoxin, Conversion factor: 1 nmol AFLA/l in cuvette = 5.739 µg/kg AFLA in sample	