INSTRUCTIONS FOR USE

aokinmycontrolAFLA M1

Order No.: MY-IC-M-31

Sample preparation with *aokin*ImmunoClean*M* columns (IAC)

aokinmycontrolAFLA M1

Analytical-kit for rapid and quantitative determination of Aflatoxin M1 (AFLA M1).

Materials:

aokin**mycontrol**AFLAM1 (Order No.: MY-IC-M-31-100)

Package content

A) *IC Consumables*:
Filter paper
aokin**ICElute **AFLA
Reaction tubes 2 mL
IC-Reservoirs (loading reservoirs) 50 mL

B) *IC Liquids:*
***aokin/C**Wash*

C) *Immunoaffinity columns:*
aokin**ImmunoClean **M AFLA M1
(Order No.: IC-M-31-100)

D) *Materials for Analytical Measurement:*
***aokin**ReactionBuffer,* Reaction buffer
aokin**mycontrol**AFLA M1, Reagent 1 (yellow),
F-AFLA M1, (for 5 analyses each)
aokin**mycontrol**AFLA M1, Reagent 2 (blue cap),
A-AFLA M1, (for 5 analyses each)

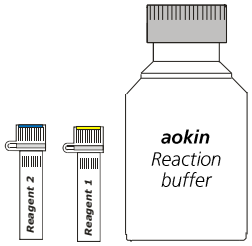


Figure 1: Reagent 1, F-AFLA M1 (yellow cap), Reagent 2, A-AFLA (blue cap) and Reaction buffer (1 L bottle)

E) *Materials for internal quality control:*
aokin**mycontrol **AFLA M1, Blank (transparent),
Blank-AFLA M1, (for zero value measurements)
aokin**mycontrol**AFLA M1, Reagent 1 (yellow),
F-AFLA M1, (for 5 analyses each)
aokin**mycontrol**AFLA M1, Reagent 2 (blue cap),
A-AFLA M1, (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.
Caution: Work with professional care.
Storage Conditions: Reagents 1, 2 and ***aokin**ImmunoClean **M AFLA M1*** columns must be stored at temperature of 2- 10°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 M1 (Order No.: MY-IC-M-31-100)

Introduction

aokin**mycontrol **AFLA M1 is a rapid and precise quantitative method for analyzing total Aflatoxin M1 (AFLA M1). It has been specifically designed and calibrated for using ***aokin**ImmunoClean **M*** columns. The sample preparation can be done in 50 minutes. ***aokin**mycontrol**AFLA M1*** is available with a calibration for skimmed milk. Please use professional care and check the accuracy by regularly analyzing reference materials (***aokin**ReferenceMatrixMaterials*) and/or standards. Participation in proficiency tests is recommended.
aokin will gladly assist you to customise this test for your specific sample type and application. Please do not hesitate to contact us.

Sample		Skimmed milk
Time required for sample preparation		50 minutes
Time required for measurement		3 minutes
Analysis		
	Measurement range [µg/kg]	
Range 1	0,01 – 0,1	

Aflatoxin M1

Aflatoxins (AFLA) are mycotoxins produced by molds such as *Aspergillus flavus* and *Aspergillus parasiticus. Aflatoxin M1* is a potent hepatotoxic and hepatocarcinogenic mycotoxin, found in milk of cows, fed on meal contaminated with *Aflatoxin B1.*

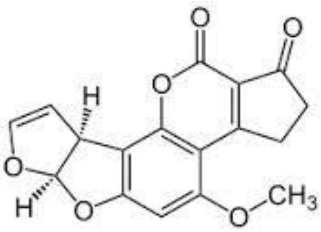


Figure 2: Chemical formula for Aflatoxin M1, $C_{17}H_{12}O_7$. Molecular weight: 328,27 g/mol

Recommended Accessories

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

	Order No.
Weighing scale, d = 0,01 g	LB-03-04
Eppendorf centrifuge, variable g-force	LB-04-04
Variable pipettes (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-31
Reaction tubes, 2mL	LB-05-02
***aokin**ICadapter*	LB-08-10-05
***aokin**VacuumAdapter*	LB-08-11
Vacuum-pump (diaphragm pump)	LB-04-10
Trap for vacuum-pump (vacuum-bottle)	LB-04-12
Vacuum manifold	LB-04-09
Magnetic stirrer plate (not heated)	LB-04-15
Magnetic stirrer (to degas)	LB-04-16-05

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 quantification range. Use volumes displayed in the ***aokin*** software.

1. Sample

Measure 60 mL of fluid milk. Centrifuge the milk sample at greater than 15.000 x g for 15 minutes (in special centrifuge tubes).
Separate fat (top) layer from defatted (skim) layer. Use defatted (skim) milk for further analysis.

2. Clean up with *aokin*ImmunoClean ***M***

a) Preparation

Attach the vacuum manifold to a pump. Attach the ***aokin***ImmunoClean ***M*** column to the vacuum manifold (or alternatively onto an ***aokin*** VacuumAdapter) and transfer ***aokin***ICWash solution onto the column. Attach the ***aokin***ICadapter to the ***aokin***ImmunoClean M column and connect it to the loading reservoir. Pipette ***aokin***ICWash into the loading reservoir.

Important: No air bubbles should be visible in the column. Let the ***aokin***ICWash solution elute, leaving only a little liquid in the reservoir and so that the ***aokin***ICadapter and ***aokin***ImmunoClean ***M*** column are still filled with liquid.

b) Loading

Load the column with 50 ml of the defattet skim milk. Adjust the vacuum so that a flow rate of approximately **1 ml/min** is produced (**Do not exceed recommended flow rates!**). The running speed can be reduced by closing the stopcock, if the sample flows thorough too fast. If the flow is too low apply vacuum (please increase the vacuum in 20 bar increments); or positive pressure by using the ***aokin***ICadapter and a syringe.

Important: Do not let the ***aokin***ImmunoClean ***M*** column run dry before, or during loading.

Important: High flow rates lead to a reduced rate of recovery.

c) Washing

Fill 10 ml ***aokin***ICWash into the loading reservoir. Pass again 10 mL ***aokin***ICWash to the column and let it run dry until air comes through column.

d) Elution

Place the column into a collection tube and centrifuge for 1 minute at 1000 x g to remove the residual liquid. Set the column into a new clean collection tube and pipette 400 µl ***aokin***ICElute ***AFLA*** into the column, close (half closed) the column after that with the lid. When closing the column, about 100 µl ***aokin***ICElute ***AFLA*** will be **pushed through the gel**. After incubation for 3 minutes, centrifuge for 1 minute at 3000 x g. The lid must remain on the column during centrifugation. The liquid is present in the collection vessel after centrifugation.

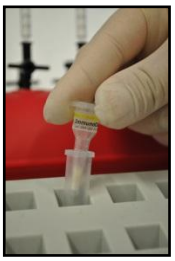


Figure 3: The column is placed into a collection tube before eluent is added, and then the column is centrifuged to remove residual liquid.

3. Analyzing

Use the eluate for analyzing in the ***aokin***spectrometer***FP470***.

Important: Gas bubbles can lead to measurement inaccuracies. It is strongly recommended to degas the reaction buffer (***aokin***ReactionBuffer) before each use. This is performed by applying vacuum and stirring for 30 min.

*Figure 4: Setup for degassing the reaction buffer (**aokin**Reaction Buffer). This consists of: vacuum pump (hose and vacuum trap, not shown) and the reaction buffer in a bottle with a magnetic stirrer rod on a magnetic stirrer plate.*

Please follow detailed instructions for spectrometer use.

This includes:

1) Place ***Reagents 1*** and ***2*** into position A6 and B6 of the sample rack of your spectrometer.
2) Fill up the ***Clean1*** solution and place a clean 2 mL vial in position A1.
3) Place an empty waste bottle in the holder. Check presence of ***Reaction buffer*** and check if tubing is below the surface.
4) Place a new cuvette with a clean stirrer into the spectrometer.

4. Quality Control

Included in the analytical kit there are following additional materials for your internal quality control: ***Reagent 1***, ***Reagent 2***, 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 perform an offset correction of the calibration based on the negative controls and a recovery correction based on the positive controls. If problem persists calibrate. Please contact the ***aokin*** team for any support needed.

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

aokinmycontrol AFLA M1 IAC Standard

Step 1: Extraction

- *Sample mass:* V_{Sample} = 50 g
- *Volume extraction solvent:* $V_{Extraction\ solvent}$ = 50 ml
- *Molar mass Aflatoxin M1:* $M_{Afla} = 328{,}2\left[\frac{g}{mol}\right] = 1{,}032\left[\frac{ng}{L}\right]$

Mycotoxin concentration in the sample extract:

$$c\left[\frac{\mu mol}{l}\right]_{Extract} = \frac{m_{Sample}[kg]}{V_{Solvent}[l]*M_{Mykotoxin}\left[\frac{g}{mol}\right]} * c\left[\frac{\mu g}{kg}\right]_{Sample} = \frac{0.05}{0.05*382{,}2*1{,}032} * c\left[\frac{\mu g}{kg}\right]_{Sample} = 0{,}00295 * c\left[\frac{\mu g}{kg}\right]_{Sample}$$

Step 2: Purification

with *aokin* IC M AFLA M1

- *Volume sample extract load to the **aokin** IC-M column:* $V_{loaded\ sample\ extract}$ = 60 ml
- *Volume "**aokin** Elute" used for the elution of the column:* V_{elute} = 0,4 ml

Mycotoxin concentration in the column eluate:

$$c\left[\frac{\mu mol}{l}\right]_{Eluate} = \frac{V_{load\ [ml]}}{V_{elute\ [ml]}} * c\left[\frac{\mu mol}{l}\right]_{Extract} = \frac{60}{0{,}4} * c\left[\frac{\mu mol}{l}\right]_{Extract} = 150 * c\left[\frac{\mu mol}{l}\right]_{Extract}$$

Step 3: Measurement

aokin FP 470 / *LHW 03*

- *Sample volume:* $V_{Column\ eluate} = V_{Sample} = 200\ \mu l$
- *Total volume in the cuvette:* $V_{Cuvette} = 2600\ \mu l$

Mycotoxin concentration in the cuvette:

$$c\left[\frac{\mu mol}{l}\right]_{Cuvette} = \frac{V_{Sample}\ [\mu l]}{V_{cuvette}\ [\mu l]} * c\left[\frac{\mu mol}{l}\right]_{Eluate} = \frac{200}{2600} * c\left[\frac{\mu mol}{l}\right]_{Eluate} = 0{,}0769 * \left[\frac{\mu mol}{l}\right]_{Eluate}$$

Conversion factor: Extraction, Purification and Measurement

It follows the conversion factor from 1 to 3 above:

$$c\left[\frac{\mu\ mol}{l}\right]_{Cuvette} = 0{,}00295 * 150 * 0{,}0769 * c\left[\frac{\mu g}{kg}\right]_{Sample} = 0{,}034 * c\left[\frac{\mu g}{kg}\right]_{Sample}$$ or

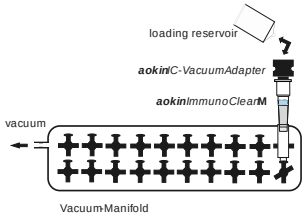
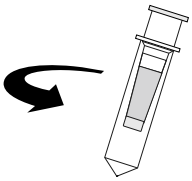
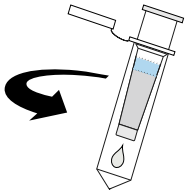
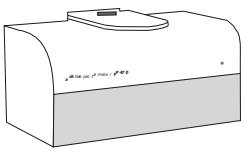
$$c\left[\frac{\mu\ g}{kg}\right]_{Sample} = \frac{1}{1.01} * c\left[\frac{nmol}{l}\right]_{Cuvette} = \mathbf{0{,}033} * c\left[\frac{nmol}{l}\right]_{Cuvette}$$

Aflatoxin M1 / standard samples:

- Recommended for milk

Procedure:

aokin*mycontrol *AFLA M1* IAC *Standard

Extraction		(Centrifuge 15 minutes at 15.000 x g, use defatted layer) 60 ml skim milk sample
Purification		set up column
		50 ml Load skimmed milk sample, 1 drop / second (slow flow rate) 40 ml wash column with ***aokin*ExtractionSolvent *AFLA M1***
		1 min centrifuge at 1000 x g (waste tube)
		**Elution:** 400 µl ***aokin*ICElute *AFLA M1*** 3 min incubation with lid on 1 min centrifuge at 3000 x g, use eluate for measurement
Measurement		**Automatic Analyse (FP470 / LHW03):** 2200 µl ***aokin*mycontrol** *Reaction buffer* 200 µl sample *(diluted 1:1 - RANGE 1)* *(diluted 1:2 - RANGE 2)* *(diluted 1:4 - RANGE 3)* 100 µl ***aokin*mycontrol *AFLA M1*** Reagent 1 100 µl ***aokin*mycontrol *AFLA M1*** Reagent 2

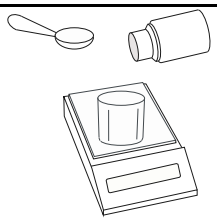
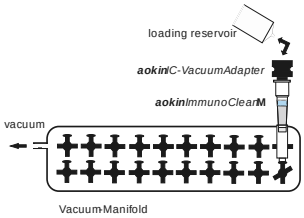
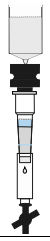
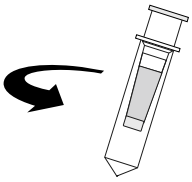
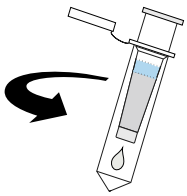
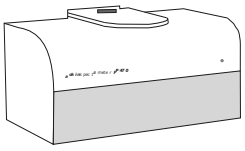
AFLA M1 = Aflatoxin M1, Conversion factor: 1 nmol AFLA M1/l in cuvette = 0,0331 µg/kg in sample

Aflatoxin M1 / dissolvable samples:

- Recommended for milk powder

Procedure:

***aokin*mycontrol *AFLA M1* IAC**
Milk powder

Extraction		**Weighing and dissolving:** 5 g sample 10 mL ***aokin*ExtractionSolvent *AFLA M1*** 35 mL dest. water
Purification		set up column
		40 ml load diluted sample, 1 drop / second (slow flow rate) 40 ml wash column with ***aokin*ExtractionSolvent *AFLA M1***
		1 min centrifuge at 1000 x g (waste tube)
		**Elution:** 400 µl ***aokin*ICElute *AFLA M1*** 3 min incubation with lid on 1 min centrifuge at 3000 x g, use eluate for measurement
Measurement		**Automatic Analyse (FP470 / LHW03):** 2200 µl ***aokin*mycontrol Reaction buffer** 200 µl sample *(diluted 1:1 - RANGE 1)* *(diluted 1:2 - RANGE 2)* *(diluted 1:4 - RANGE 3)* 100 µl ***aokin*mycontrol *AFLA M1*** Reagent 1 100 µl ***aokin*mycontrol *AFLA M1*** Reagent 2

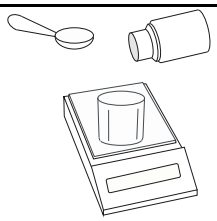
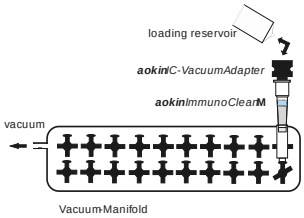
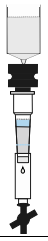
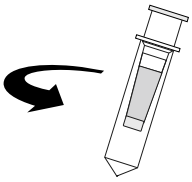
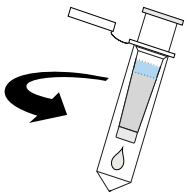
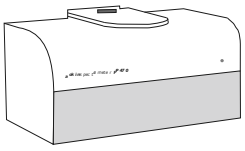
AFLA M1 = Aflatoxin M1, Conversion factor: 1 nmol AFLA M1/l in cuvette = 0,384 µg/kg in sample

Aflatoxin M1 / dissolvable samples:

- Recommended for galactose

Procedure:

aokin*mycontrol *AFLA M1* IAC *Dissolvables

Extraction		**Weighing and dissolving:** 5 g sample 25 mL *aokinExtractionSolvent AFLA M1* 60 mL dest. water
Purification		set up column
		85 ml load diluted sample, 1 drop / second (slow flow rate) 20 ml wash column with *aokinExtractionSolvent AFLA M1*
		1 min centrifuge at 1000 x g (waste tube)
		**Elution:** 400 µl *aokinICElute AFLA M1* 3 min incubation with lid on 1 min centrifuge at 3000 x g, use eluate for measurement
Measurement		**Automatic Analyse (FP470 / LHW03):** 2200 µl *aokinmycontrol Reaction buffer* 200 µl sample *(diluted 1:1 - RANGE 1)* *(diluted 1:2 - RANGE 2)* *(diluted 1:4 - RANGE 3)* 100 µl *aokinmycontrol AFLA M1* Reagent 1 100 µl *aokinmycontrol AFLA M1* Reagent 2

AFLA M1 = Aflatoxin M1, Conversion factor: 1 nmol AFLA M1/l in cuvette = 0,1 µg/kg in sample