INSTRUCTIONS FOR USE

aokinmycontrol AFLA SPE

Order No.: MYS-QC-03

Quantitative determination of **Aflatoxin total**

Includes sample preparation with *aokinQuickClean* columns (SPE) designed for use with ***aokin* FP analyzer**.

Package content:

Order No. MYS-QC-03
aokin**mycontrol* ***AFLA SPE

Order No	**Material**	**Content**	**Application**	**Storage**
MYS-QC-03-20 for 20 measurements	***aokin**mycontrol* ***AFLA SPE*** Kit for quantitative determination of Aflatoxin (AFLA)	***aokin**ExtractionSalt* ***AFLA/OTA*** ①, 1 pouch for 20 preparations ***aokin*** *Easy Extract Liquid* ***AFLA*** ②, 1 vial *sticker for* ***aokin*** *EasyExtractBuffer* ***AFLA*** ② ***aokin**QuickClean* ***AFLA*** ③, 40 units ***aokin*** *ReactionBuffer* ④, 1 bottle ***aokin**mycontrol**AFLA** Additive* ⑤, 2 units ***AFLA*** *Reagent* ⑤, 1 unit ***AFLA*** *Tracer* ⑤, 1 unit ***AFLA*** *positive control, Standard* ***AFLA B1*** ⑤, 50µL, 1 unit	Measurement on ***aokin* FP analyzer**	Refrigerated Do not freeze
MYS-QC-03-100 for 100 measurements	***aokin**mycontrol* ***AFLA SPE*** Kit for quantitative determination of Aflatoxin (AFLA)	***aokin**ExtractionSalt* ***AFLA/OTA*** ①, 1 unit for 100 preparations ***aokin*** *Easy Extract Liquid* ***AFLA*** ②, 1 bottle *4x sticker for* ***aokin*** *EasyExtractBuffer* ***AFLA*** 2x ***aokin**QuickClean* ***AFLA*** ③, 100 units ***aokin*** *EasyReaction Buffer Component A* ④Ⓐ, 1 unit ***aokin*** *EasyReaction Buffer Component B* ④Ⓑ, 1 unit *sticker for* ***aokin*** *EasyReactionBuffer* ④ ***aokin**mycontrol**AFLA** Additive,* 10 units ⑤ ***AFLA*** *Reagent* ⑤, 5 units ***AFLA*** *Tracer* ⑤, 5 units ***AFLA*** *positive control, Standard* ***AFLA B1*** ⑤, 50µL, 5 units	Measurement on ***aokin* FP analyzer**	Refrigerated Do not freeze

Analytical-kit for rapid and quantitative determination of Aflatoxin (AFLA) in corn and other grains.

Materials

All materials provided are precisely weighed and calibrated. Control of the volume and concentration of the individual solutions are essential for the precision of the analysis.

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.

Preparation of buffers:

MYS-QC-03-20 for 20 measurements:
Mix 190 mL destilled or deionized water with 10 mL of ***aokin*** *Easy Extract Liquid* ***AFLA*** ② and 800 mL of Methanol = ***aokin*** *EasyExtractBuffer* ***AFLA*** ②. Prepare the solution and label the container with the sticker ***aokin*** *EasyExtractBuffer* ***AFLA*** ② included in the kit, on which you can note the preparation date.
aokin *ReactionBuffer* ④ included in the kit has to be brought to room temperature (best 18-26°C) for usage.

MYS-QC-03-100 for 100 measurements:
Mix 190 mL destilled or deionized water with 10 mL of ***aokin*** *Easy Extract Liquid* ***AFLA*** ② and 800 mL of Methanol = ***aokin*** *EasyExtractBuffer* ***AFLA*** ②. Prepare 4x the solution and label the container with the sticker ***aokin*** *EasyExtractBuffer* ***AFLA*** ② included in the kit, on which you can note the preparation date.
The complete content of the *EasyReaction Buffer Component A* ④Ⓐ plus the *EasyReaction Buffer Component B* ④Ⓑ needs to be dissolved in a total of 500 mL destilled or deionized water = ***aokin*** *EasyReactionBuffer* ④. Prepare the solution and label the container with the sticker ***aokin*** *EasyReactionBuffer* ④ included in the kit, on which you can note the preparation date. ***aokin*** *EasyReactionBuffer* ④ has to be brought to room temperature (best 18-26°C) for usage.

Storage and stability:

The diluted ***aokin*** *EasyExtractBuffer* ***AFLA*** ② is stable for 12 months without refrigeration (15° - 30°C).

aokin *ReactionBuffer* ④ is stable for 12 months without refrigeration (15° - 30°C).

Kit reagents (***AFLA*** *Reagent* ⑤, ***AFLA*** *Tracer* ⑤, ***AFLA*** *Additive* ⑤, *positive control* ⑤) must be stored in a refrigerator (2-10°C). ***AFLA*** *positive control* is stable after dilution for only 1 day.

Any other components can be stored at ambient temperature.

Do not use expired or contaminated components, or components from other kits. Do not mix components from different manufactured lots. A certificate of analysis (COA) is available upon request at info@aokin.com.

Materials and instrumentation required but not provided:

***aokin* FP analyzer**, 10x75 mm borosilicate glass test tubes for ***aokin* FP analyzer**, Micro-pipette: 10 – 100 µL, 100-1000 µL and related pipette tips, 2 mL collection tubes, tube vortex mixer, centrifuge, timer, deionized water for buffer reconstitution, methanol.

Alternative to the ***aokin* FP analyzer** other FP single tube readers or FP microplate readers can be used. Depending on instrumentation the applied volumes need adjustment. Black flat bottom microtiter plates are recommended for use with FP microplate readers.

Introduction

aokin*mycontrol* ***AFLA SPE*** is a rapid method for quantifying Aflatoxin (AFLA). It has been specifically designed and calibrated for the analysis of corn and includes a sample preparation with matrix removal columns (***aokin****QuickClean* ***AFLA***). Samples in the µg/kg range (µg/kg = ppb) can be analysed for AFLA in under 10 minutes.

aokin*mycontrol* ***AFLA SPE*** is available with a calibration, which has been validated for wheat products and others like animal feed. Daily checks of negative control and positive control (either liquid standard or reference matrix extract) are required to ensure reliable results.

FPA technology works on clear homogeneous liquids without particles or emulsions, therefore avoid practices that may contaminate the test controls, test buffer, or test reagents. ***aokin*** will gladly assist you customizing the test for your specific sample type and application. Please do not hesitate to contact us.

Polarization readings are affected by temperature. Polarization readings decrease by around 3 mP (millipolarization units) for a temperature increase of 4°C.
The method is best used at constant temperatures between controls and sample measures. If a temperature change of >10°C is observed, a new calibration is recommended.

Health:

All materials in this kit should be treated as any other laboratory chemicals. Avoid ingestion, eye contact and other potential detrimental exposure. A material safety data sheet (MSDS) is available upon request.

Negative controls:

Negative control should be run in triplicate at regular intervals with the single tube assay or on every microtiter plate, respectively. Avoid temperature variations of more than 1°C during one determination. Mix well with a vortexer and wait 60 s before taking the value. Be careful to avoid bubbles when pipetting into microtiter plates.

Negative control AFLA SPE = **4 mL of prepared *aokin* *EasyExtractBuffer* *AFLA*** ② **diluted with 6.5 mL of destilled or deionized water** (exact same ratio as in protocol)

Positive controls:

Positive controls are recommended as an additional measure to adjust the calibration for small temperature changes. Reference matrix materials are best used to determine the recovery rate and to do a recovery rate correction if needed.

Positive control AFLA SPE ⑤ = Standards diluted in prepared negative control AFLA SPE

Dilute included **Aflatoxin B1 300 ng/mL in Acetonitrile** with prepared Negative control AFLA SPE (Dilutionfactor **1:120** -> example: add 10 µL of **Aflatoxin B1 300 ng/mL in Acetonitrile** to 1190 µL Negative control AFLA SPE (4 mL of prepared ***aokin*** *EasyExtractBuffer* ***AFLA*** ② diluted with 6.5 mL of destilled or deionized water). Use 50 to 350 µL within the assay, fill up with prepared Negative control AFLA SPE for overall 450 µL sample volume.

(Example: 200 µL of **1:120 diluted Aflatoxin B1 300 ng/mL** + 250 µL Negative control AFLA SPE = 1,11 ng/mL Aflatoxin B1 within the assay)

Aflatoxins

Aflatoxins are mycotoxins produced by molds such as *Aspergillus flavus* and *Aspergillus parasiticus.* Aflatoxin B1 (AFLA) 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.

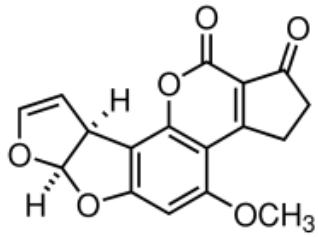


Figure 1: Chemical formula for Aflatoxin B1 ($C_{17}H_{12}O_6$, Molecular weight: 312.3 g/mol)

Standard sample preparation for corn (Page 8/8)

1. Preparation

1.1. Allow ***aokin****ReactionBuffer* ④ to reach room temperature (15°C to 30°C) before use.

1.2. Prepare buffers and solutions:
aokin *EasyExtractBuffer* ***AFLA*** ② = ***aokin*** *Easy Extract Liquid* ***AFLA*** ② dissolved in deionized water and Methanol.
Negative control AFLA SPE = prepared ***aokin*** *EasyExtractBuffer* ***AFLA*** ② diluted with deionized water.
Positive control AFLA SPE = AFLA Standard ⑤ diluted in prepared Negative control AFLA SPE.

2. Instrument adjustment
Instruments must be set up and calibrated according to manufacturer's specifications. For detailed information, please consult instrument manual and contact technical support.

3. Sample collection, grinding and mixing
The analysis sample is collected, ground, and mixed/homogenised according to an approved procedure. For convenience volumes may be ground and mixed by using a knife-blender.

4. Weighing and extraction
Weigh 15 g of your sample, add one spoon (1.5 g) of ***aokin****ExtractionSalt* ***AFLA*** ① and 35 mL ***aokin*** *EasyExtractBuffer* ***AFLA*** ② directly into an extraction beaker. Preferentially the exact volume is applied using a dispensette

Extract sample by blending for 3.5 minutes at high speed. 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). Preferably use a preprogrammed timer (***aokin***watchbox) to conveniently and automatically complete this extraction protocol.

5. Filtration or centrifugation
Filter the raw extract through a fluted filter. Discard the filter cake and use the filtrate. Alternatively centrifuge the raw extract and use the supernatant.

6. Use of *aokin*QuickClean column
Place an ***aokin****QuickClean* ***AFLA*** ③ in a 2 mL collection tube and add 900 µL of the filtrate/supernatant (*Figure 2*). Place it in the centrifuge and spin for 3 minutes at 5000 x g. Place second ***aokin****QuickClean* ***AFLA*** ③ column in a 2 mL collection tube and add ≥ 650 µL of the purified filtrate from the first column. Place it again in the centrifuge and spin for 3 minutes at 5000 x g or until all liquid passed the column.

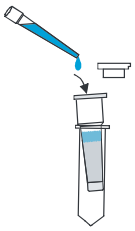


Figure 2: Pipetting of the extract onto the ***aokin****QuickClean column*

7. Dilution

Pipet 400 µL of the purified filtrate and 650 µL of deionized water and mix it well. This step is **equal to a 1:2.6 dilution**. In case a precipitation is visible centrifuge with maximum g-force (> 10000 x g) for 3 minutes. Transfer the supernatant into a clean tube. Your sample is now ready for analysis.

8. Introduction of general testing procedure

8.1. Pipette 515 µl of ***aokin****ReactionBuffer* ④ into the test tube.
8.2. Pipette 100 µl of ***aokin****mycontrol****AFLA*** *Additive* (***BLACK*** *cap*) ⑤ into the test tube.
8.3. Add 450 µL of clear sample extract (if necessery fill up with Negative control AFLA SPE solution if sample extract is too concentrated, example: 200 µL sample + 250 µL Negative control AFLA SPE for overall 450 µL sample volume) or a control into the test tube.

Note: Run negative controls in triplicate, and positive control and samples as duplicates for best performance.

8.4. Add 15 µL of ***AFLA*** *Reagent* (***GREEN*** *cap)* ⑤ into the test tube.
8.5. Mix well/vortex (without spilling).
8.6. Obtain background measurement readings of all samples and controls. Wait a minimum of 1 minute and a maximum of 15 minutes between mixing and reading.
8.7. Add 20 µL of ***AFLA*** *Tracer* (***WHITE*** cap) ⑤ into the first test tube.
8.8. Mix well.
8.9. Incubate for 60 seconds.
8.10. Obtain tracer measurement of the sample.
8.11. Repeat steps 8.6 to 8.9 for all test tubes.

Note: Include one set of control after each hour of testing, or on each microtiter plate. Performing measurements of negative controls regularly will ensure the accuracy of your determinations.

9. Data acquisition and analysis

9.1. Mix and Read Process

9.1.1. Mix buffer, sample and reagent in exact volumes and at exactly specified time intervals. Read background fluorescence in horizontal and vertical direction.
9.1.2. Add tracer, incubate for exactly the specified time. Read emission values in horizontal and vertical direction.

9.2. Background information

The fluorophore is excited with polarized light, depending on the rotational velocity of the fluorophore (or the fluorophore antibody complex) the emission will also be polarized. The emission intensities using horizontally directed and vertically directed polarization filters are detected for the background and for the reaction. Followed by an automatic calculation of polarization from the value changes in vertical and horizontal direction.
$P = (\Delta H - \Delta V)/ (\Delta H + \Delta V)$.

9.3. Software: direct use of excel worksheet - *aokin mycontrol* version of software – alternatively use user interface

Advice: Use a copy of the empty original excel worksheet to do all measurements of one series. When you start with a new series including negative controls, positive controls and sample analysis measurements, use a new copy of the empty original worksheet saved previously with the name of your series and the date.

Note:

- Connect computer with ***aokin*** FP analyser
- Install the ***aokin*** software
- One excel row = one experiment
- Green cells can be written into
- Yellow cells are for measuring: highlight a cell in the correct line and press F3
- Brown cells are for information only
- Light green cells contain evaluations = calculations and end results

10. Daily Check I:

a. Use negative control (= 4 mL of prepared *aokin* *EasyExtractBuffer* ***AFLA* ② diluted with 6.5 mL of destilled or deionized water) in Step 8.3**
b. Software: Choose *Daily Check I* in the user interface or alternatively *Offset at given T°C* Sheet in particular excel version of software

10.1. Pipette 515 µl of ***aokin****ReactionBuffer* ④ into a test tube.
10.2. Pipette 100 µl of ***aokin****mycontrol****AFLA*** *Additive* (***BLACK*** *cap*) ⑤ into the test tube.
10.3. Add 450 µL of a negative control into the test tube. The negative control for AFLA SPE is prepared by mixing 4 mL of prepared ***aokin*** *EasyExtractBuffer* ***AFLA*** ② with 6.5 mL of destilled or deionized water.
10.4. Add 15 µL of ***AFLA*** *Reagent (****GREEN*** *cap)* ⑤ into the test tube.
10.5. Mix well/vortex (without spilling).
10.6. Obtain background measurement reading. Wait a minimum of 1 minute and a maximum of 15 minutes between mixing and reading.
10.7. Add 20 µL of ***AFLA*** *Tracer (****WHITE*** cap) ⑤ into the first test tube.
10.8. Mix well.
10.9. Incubate for 60 seconds.
10.10. Obtain tracer measurement

Note: Run negative controls in triplicate.

11. Daily Check II-A:
a. Use liquid positive control in Step 8.3
b. Software: Choose *Daily Check II, Liquid standard* in the user interface or *Recovery rate at given T°C* Sheet in particular excel version of software

11.1. Pipette 515 µl of ***aokin****ReactionBuffer* ④ into a test tube.
11.2. Pipette 100 µl of ***aokin****mycontrol****AFLA*** *Additive* (***BLACK*** *cap*) ⑤ into the test tube.
11.3. Add 450 µL of a positive control into the test tube. The positive control is a liquid standard diluted in prepared Negative control AFLA SPE.
Respectively dilute included **Aflatoxin B1 300 ng/mL in Acetonitrile** with prepared Negative control AFLA SPE (Dilutionfactor **1:120** -> example: add 10 µL of **Aflatoxin B1 300 ng/mL in Acetonitrile** to 1190 µL Negative control AFLA SPE (4 mL of prepared ***aokin*** *EasyExtractBuffer* ***AFLA*** ② diluted with 6.5 mL of destilled or deionized water). Use 50 to 350 µL within the assay, fill up with prepared Negative control AFLA SPE for overall 450 µL sample volume.
(Example: 200 µL of **1:120 diluted Aflatoxin B1 300 ng/mL** + 250 µL Negative control AFLA SPE = 1,11 ng/mL Aflatoxin B1 within the Assay)
11.4. Add 15 µL of ***AFLA*** *Reagent (****GREEN*** *cap)* ⑤ into the test tube.
11.5. Mix well/vortex (without spilling).
11.6. Obtain background measurement reading. Wait a minimum of 1 minute and a maximum of 15 minutes between mixing and reading.
11.7. Add 20 µL of ***AFLA*** *Tracer (****WHITE*** cap) ⑤ into the first test tube.
11.8. Mix well.
11.9. Incubate for 60 seconds.
11.10. Obtain tracer measurement

Note: Run positive controls in duplicate.

12. Alternative Daily Check II-B:
a. Use of final extract of a reference matrix material as positive control in Step 8.3
b. Software: Choose *Daily Check II, Reference Matrix* in the user interface or *Recovery rate at given T°C* Sheet in particular excel version of software

12.1. Choose a reference matrix sample most similar to your samples.
Example: In case you analyse wheat use a wheat reference matrix sample containing a known amount of mycotoxin.
12.2. Prepare the reference matrix sample according to Steps 4, 5, 6, 7.
12.3. Pipette 515 µl of ***aokin****ReactionBuffer* ④ into a test tube.
12.4. Pipette 100 µl of ***aokin****mycontrol****AFLA*** *Additive* (***BLACK*** *cap*) ⑤ into the test tube.
12.5. Add 450 µL of the Reference Matrix extract into the test tube.
12.6. Add 15 µL of ***AFLA*** *Reagent (****GREEN*** *cap)* ⑤ into the test tube.
12.7. Mix well/vortex (without spilling).
12.8. Obtain background measurement reading. Wait a minimum of 1 minute and a maximum of 15 minutes between mixing and reading.
12.9. Add 20 µL of ***AFLA*** *Tracer (****WHITE*** cap) ⑤ into the first test tube.

12.10. Mix well.
12.11. Incubate for 6 minutes.
12.12. Obtain tracer measurement

Note: Run positive controls in duplicate. Repeat with a different dilution (8.3), if needed.

13. Testing procedure – Sample:
a. Use sample extract in Step 8.3
b. Software: Choose *Analysis* in the user interface or *Analysis at given T°C* Sheet in particular excel version of software

13.1. Pipette 515 µl of ***aokin****ReactionBuffer* ④ into the test tube.
13.2. Pipette 100 µl of ***aokin****mycontrol****AFLA*** *Additive* (***BLACK*** *cap*) ⑤ into the test tube.
13.3. Add 450 µL of clear sample extract or a control into the test tube.
13.4. Add 15 µL of ***AFLA*** *Reagent (****GREEN*** *cap)* ⑤ into the test tube.
13.5. Mix well/vortex (without spilling).
13.6. Obtain background measurement readings of all samples and controls. Wait a minimum of 1 minute and a maximum of 15 minutes between mixing and reading.
13.7. Add 20 µL of ***AFLA*** *Tracer (****WHITE*** cap) ⑤ into the first test tube.
13.8. Mix well.
13.9. Incubate for 60 seconds.
13.10. Obtain tracer measurement of the first sample.
13.11. Repeat steps 13.7 to 13.10 for all test tubes.

Note: Run samples as duplicates. Repeat with a different dilution (8.3), if needed.

14. Test validation

14.1. The mean negative control must read between 150 and 220 mP.
14.2. The positive control must always read lower than the negative control. Expected values of the positive control are 10 mP to 120 mP lower than the negative control.
14.3. If the negative or positive control produce values outside of the expected values, the instrument should be recalibrated using the procedures described in the instrument manual.
14.4. Check the accuracy of your measurements by regularly analysing reference materials (e.g. ***aokin****ReferenceMatrixMaterials*), or other verified materials with known amounts of contaminants. Participation in proficiency tests is recommended as good laboratory practice.

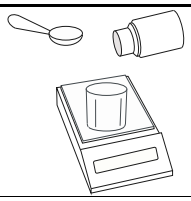
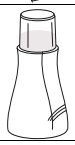
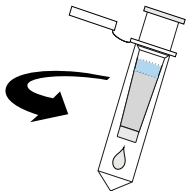
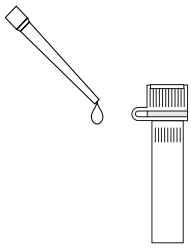
Recommendation: If you notice erroneous measurement values, change the test tube and repeat the measurement after performing measurement of controls. Check that the liquid in the tube is not cloudy and that there are no bubbles after mixing.

Note: Repeat the daily checks after each hour of testing, or on each microtiter plate. Performing measurements of controls regularly will ensure the accuracy of your determinations. The results of the daily checks are used to ensure the analytical performance.

Be aware: The general composition of controls and samples should always be identical in salt concentration, pH and solvent ratio.

Standard

Sample preparation for **corn, corn flour**

Extraction		**Weighing:** 15 g sample 1.5 g ***aokin**ExtractionSalt **AFLA*** ① 35 mL ***aokin** EasyExtractBuffer **AFLA*** ②
		**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 **AFLA*** ③ 3 min centrifuge at 5000 x g **2. column:** ≥ 650 µL filtrate of the 1st ***aokin**QuickClean* ③ on 2nd ***aokin**QuickClean* ③ column 3 min centrifuge at 5000 x g or until all liquid passed column use 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	Mix and read	**Quantification** add into tube: 515 µL ***aokin** ReactionBuffer* ④ 100 µL ***aokin**mycontrol**AFLA** Additive **BLACK*** ⑤ 450 µL sample 15 µL ***AFLA** Reagent **GREEN*** ⑤ mix well measure background 20 µL add ***AFLA** Tracer **WHITE*** ⑤ mix well, incubate for 60 seconds measure tracer