INSTRUCTIONS FOR USE

aokin mycontrol ZON

Order No.: MY-QC-01

Sample preparation with aokinQuickClean columns (SPE)

aokinmycontrolZON

Analytical-kit for rapid and quantitative determination of Zearalenone (ZON).

Materials

aokinmycontrolZON (Order No.: MY-QC-01-100)

Package content

A) *Materials for sample preparation:*
aokinExtractionSolventZON, Extraction solution
aokinExtractionSalt ZON + spoon
aokinQuickCleanZON, centrifuge columns
aokinmycontrolZON Precipitation buffer (white cap)
Filter paper
Reaction tubes 2 mL

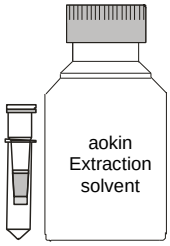


Figure 1: aokinQuickClean *column with reaction tube and* Extraction solvent *(1 L bottle)*

B) *Materials for analytical measurement:*
aokinReactionBuffer, Reaction buffer
aokinmycontrolZON, Reagent 1 (yellow cap), F-ZON, (for 5 analyses each)
aokinmycontrolZON, Reagent 2 (red cap), A-ZON, (for 5 analyses each)

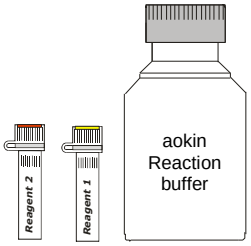


Figure 2: Reagent 1, F-ZON (yellow cap), Reagent 2, A-ZON (red cap) and Reaction buffer (1 L bottle)

C) *Materials for internal quality control:*
aokin*mycontrol* ZON, negative control ZON (transparent), for zero value measurements)
aokinmycontrol ZON, Reagent 1 (yellow cap), F-ZON, (for 5 analyses each)
aokinmycontrol ZON, Reagent 2 (red cap), A-ZON, (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: The extraction solvent may contain methanol. Work with professional care.
Storage Conditions: Reagents 1 and 2 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:
aokinmycontrolZON (Order No.: MY-QC-01-100)

Introduction

aokinmycontrol is a rapid and precise quantitative method for analyzing zearalenone (ZON). It has been specifically designed and calibrated for the analysis of food and feed and includes a sample preparation with mixed phase extraction (SPE) columns. Samples in the µg/kg range (ppb = parts per billion range) can be analysed for ZON in 12 minutes.
aokinmycontrolZON is available with a calibration, which has been validated for grain and other food products. Please use professional care and check the accuracy by regularly analyzing reference materials (e.g. aokinReferenceMatrix Materials) and/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	grain, food, feed
Time required for sample preparation	9 minutes
Time required for measurement	3 minutes

Analysis	
	Measurement range [µg/kg]
Range 1	6 – 90
Range 2	12 – 180
Range 3	28 – 419,4

Zearalenone

Zearalenone (ZON) is also known as ZEA, RAL and F-2 mycotoxin. ZON is the primary toxin causing infertility, abortion or other breeding problems in swine. It is heat-stable and is found in cereal crops, such as maize, barley, oats, wheat, rice, and sorghum. As a consequence, it is recommended to monitor ZON content in food and feed products.

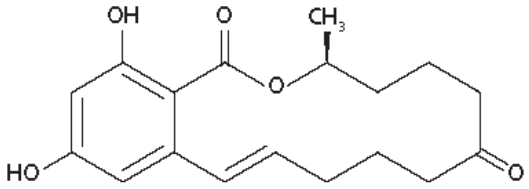


Figure 3: Chemical formula for Zearalenone $C_{18}H_{22}O_5$
Molecular weight: 318,36 g/mol

Recommended Accessories

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

	Order No.:
aokinextractor (food blender)	EX-07-06
aokinwatchbox (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 pipettes (1000 µl)	LB-04-05-1000
Pipette tips (1000 µl)	LB-04-08-1000
Funnels	LB-05-04
Dispensette	LB-08-01
aokinReferenceMatrixMaterial	RMM-01

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 determina-tion. 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***, 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 homogenised according to an approved procedure. Small samples may be ground using the ***aokin****extractor.*

3. Weighing and extraction

Weigh 15 g of your sample, add one spoon (1,5 g) of *aokinExtractionSalt* ZON and 30,4 g extraction solution (35 ml aokinExtractionSolvent ZON 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 extrac-tion protocol.

Figure 5: Extracting with the aokinextractor *(blender)*

4. 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

5. Use of aokinQuickClean column

Place an aokinQuickClean **ZON** column in a collection tube and add 400 µl of the filtrate (Figure 7). Place it in the centrifuge and spin for 3 minutes at 3000 x g.

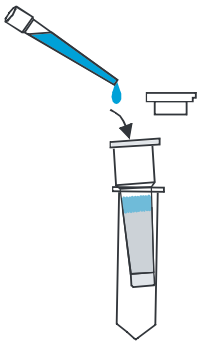


Figure 7: Pipetting of the extract onto the aokinQuickCleanZON *column*

6. Precipitation

Add 140 µl of column-filtrate into the aokinmycontrolZON precipitation buffer (white cap) and mix it well. In case a precipitation is visible, centrifuge at maximum g-force (> 10.000 x g) for 2 minutes.

Transfer 1 mL supernatant into a clean tube. Your sample is now ready for analysis.

7. Analyzing

Use supernatant of precipitation for analyzing in the ***aokin****spectrometer****FP470.***

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

This includes:

1) Place *Reagents 1* and *2* into position A6 and B6 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 ZON*-glass container, filled with negative control ZON solution = 1,4 mL aokinExtractionSolvent ZON + 17,2 mL aokinmycontrolZON precipitation buffer 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 in the holder. Check presence of degased 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)

***aokin**mycontrol ZON*
Standard

Step 1: Extraction

- *Sample mass:* m $_{Sample}$ = 15 g
- *Volume extraction solvent:* V $_{Extraction\ solvent}$ = 35 ml
- *Molar mass Zearalenone:* $MW_{ZON} = 318{,}36\ \left[\frac{g}{mol}\right]$

Mycotoxin concentration in the sample extract:

$$\boldsymbol{c}\left[\frac{\boldsymbol{\mu mol}}{\boldsymbol{l}}\right]_{\boldsymbol{Extract}} = \frac{m_{Sample}[kg]}{V_{Solvent}[l] * MW_{Mykotoxin}\left[\frac{g}{mol}\right]} * c\left[\frac{\mu g}{kg}\right]_{Sample} = \frac{0.015}{0.035 * 318{,}36} * c\left[\frac{\mu g}{kg}\right]_{Sample} = 0.0013462 * c\left[\frac{\mu g}{kg}\right]_{Sample}$$

Step 2: Purification

with ***aokin*** *QC ZON*

- *Volume sample extract load to the **aokin** QC column:* *V* $_{loaded\ sample\ extract}$ = 0,4 ml
- *Volume eluate from the **aokin** QC column:* *V* $_{elute}$ = 0,4 ml

Mycotoxin concentration in the column eluate:

$$\boldsymbol{c}\left[\frac{\boldsymbol{\mu mol}}{\boldsymbol{l}}\right]_{\boldsymbol{Eluate}} = \frac{V_{load\ [ml]}}{V_{elute\ [ml]}} * c\left[\frac{\mu mol}{l}\right]_{Extract} = \frac{0.4}{0.4} * c\left[\frac{\mu mol}{l}\right]_{Extract} = 1 * c\left[\frac{\mu mol}{l}\right]_{Extract}$$

Step 3: Dilution

- *Volume Eluate:* *V* $_{eluate}$ = 0,14 ml
- *Total volume:* *V* $_{total}$ = 1,86 ml

$$\boldsymbol{c}\left[\frac{\boldsymbol{\mu mol}}{\boldsymbol{l}}\right]_{\boldsymbol{Diluted}} = \frac{V_{eluate\ [ml]}}{V_{total\ [ml]}} * c\left[\frac{\mu mol}{l}\right]_{Extract} = \frac{0.14}{1.86} * c\left[\frac{\mu mol}{l}\right]_{Extract} = 0{,}0753 * c\left[\frac{\mu mol}{l}\right]_{Extract}$$

Step 4: Measurement

aokin FP 470 / *LHW 03*

- *Sample volume:* $V_{Column\ eluate} = V_{Sample} = 700\ \mu l$
- *Total volume in the cuvette:* $V_{Cuvette} = 2600\ \mu l$

Mycotoxin concentration in the cuvette:

$$\boldsymbol{c}\left[\frac{\boldsymbol{\mu mol}}{\boldsymbol{l}}\right]_{\boldsymbol{Cuvette}} = \frac{V_{Sample}\ [\mu l]}{V_{cuvette}\ [\mu l]} * c\left[\frac{\mu mol}{l}\right]_{Eluate} = \frac{700}{2600} * c\left[\frac{\mu mol}{l}\right]_{Eluate} = 0.269 * \left[\frac{\mu mol}{l}\right]_{Eluate}$$

Conversion factor: Extraction, Purification and Measurement

It follows the conversion factor from 1 to 4 above:

$$\boldsymbol{c}\left[\frac{\mu\ \boldsymbol{mol}}{\boldsymbol{l}}\right]_{\boldsymbol{Cuvette}} = 0{,}0013462 * 1 * 0{,}0753 * 0.269 * c\left[\frac{\mu g}{kg}\right]_{Sample} = 0.00002727 * c\left[\frac{\mu g}{kg}\right]_{Sample} \quad \text{or}$$

$$\boldsymbol{c}\left[\frac{\mu\ \boldsymbol{g}}{\boldsymbol{kg}}\right]_{\boldsymbol{Sample}} = \frac{1}{0.02727} * c\left[\frac{nmol}{l}\right]_{Cuvette} = 36.67 * \boldsymbol{c}\left[\frac{\boldsymbol{nmol}}{\boldsymbol{l}}\right]_{\boldsymbol{Cuvette}}$$

Dilution scheme of the standard solution sent for control purposes:

ZON Cal 6,78 a.u.

Calibration:

Dilution scheme (example for standard experimental setup of 700 µl sample into 2600 µl of total cuvette volume):

ZON Cal 6,78 a.u.

↓ **1:13,3 diltution** = ZON Cal 6,78 a.u. (140 µl) in ZON Dilution Buffer (1720 µl)

(→ **6.78 nM in cuvette**)

↓ **1:2 diltution** in *negative control ZON*

(→ **3.39 nM in cuvette**)

↓ **1:2 diltution** in *negative control ZON*

(→ **1.695 nM in cuvette**)

↓ **1:2 diltution** in *negative control ZON*

(→ **1.85 nM in cuvette**)

negative control ZON
(→ **0.00 nM in cuvette**)

Positive control:

Dilution scheme (example for standard experimental setup of 700 µl sample into 2600 µl of total cuvette volume):

ZON Cal 6,78 a.u.

↓ **1:13,3 diltution** = ZON Cal 6,78 a.u. (140 µl) in ZON Dilution Buffer (1720 µl)

700 µl in RANGE 1
(25.18 nM in vial ----------→ 6.78 nM in cuvette
in calculated volume for solid sample → 248.62 µg/kg)

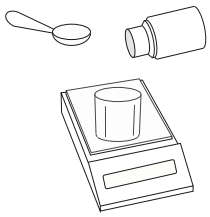
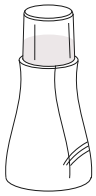
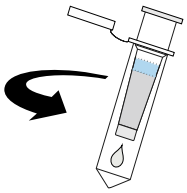
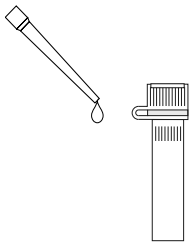
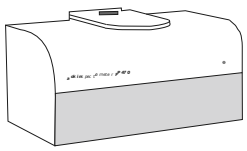
350 µl in RANGE 2
(12.59 nM in vial ----------→ 3.39 nM in cuvette
in calculated volume for solid sample → 124.31 µg/kg)

150 µl in RANGE 3
(5.39 nM in vial ----------→ 1.45 nM in cuvette
in calculated volume for solid sample → 53.24 µg/kg)

Zearalenone / standard samples:

aokinmycontrol **ZON**
Standard

Procedure:

Extraction		**Weighing:** 15 g sample 1,5 g ***aokin**ExtractionSalt **ZON*** 35 mL ***aokin**ExtractionSolvent **ZON***
		**Extraction:** 3,5 min mixing with ***aokin**watchbox*
		**Filtration:** collect filtrate (discard filter cake)
Purification		**SPE-Filtration:** 400 µL filtrate on ***aokin**QuickClean* column 3 min centrifuge at 3.000 x g
Precipitation		**Precipitation:** 140 µl column filtrate into *Precipitation buffer* (transparent cap) 2 min centrifuge at > 10.000 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* 1700 µl ***aokin** Reaction buffer* 700 µl sample *(diluted 1:1 - RANGE 1, 700µl sample)* *(diluted 1:2 - RANGE 2, 350µl sample)* *(diluted 1:4.67 - RANGE 3, 150µl sample)* 100 µl ***aokin**mycontrol **ZON** Reagent 1* 100 µl ***aokin**mycontrol **ZON** Reagent 2*

ZON = Zearalenone, Conversion factor: 1 nmol ZON/l in cuvette = 36,67 µg/kg in sample