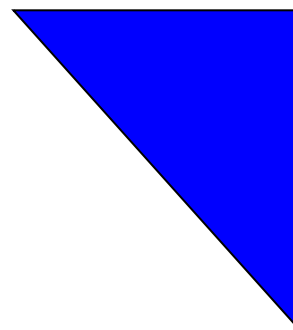


# INSTRUCTIONS FOR USE



## **aokin** mycontrol **DON**

Order No.: MY-QC-02

### Sample preparation with aokinQuickClean columns (SPE)



## 

Analytical-kit for rapid and quantitative determination of Deoxynivalenol (DON).

### Materials

aokinmycontrolDON (Order No.: MY-QC-02-100)

Package content

A) *Materials for sample preparation:*

aokinExtractionSolventDON, Extraction solution

aokinQuickCleanDON, centrifuge columns

Filter paper

Reaction tubes 2 mL

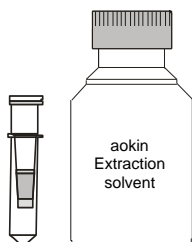


Figure 1: aokinQuickClean column with reaction tube and Extraction solvent (2,5 L bottle)

B) *Materials for analytical measurement:*

aokinReactionBuffer, Reaction buffer

aokinmycontrolDON, Reagent 1 (red cap), F-DON,  
(for 5 analyses each)

aokinmycontrolDON, Reagent 2 (blue cap), A-DON,  
(for 5 analyses each)

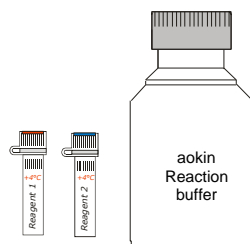


Figure 2:  
Reagent 1 (red cap),  
Reagent 2 (blue cap) and  
Reaction buffer (1 L bottle)

C) *Materials for internal quality control:*

aokinmycontrolDON, negative control DON  
(transparent), (for zero value measurements)

aokinmycontrolDON, Reagent 1 (red cap), F-DON,  
(for 5 analyses each)

aokinmycontrolDON, Reagent 2 (blue cap), A-DON,  
(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.

**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:

aokinmycontrolDON (Order No.: MY-QC-02-100)

### Introduction

aokinmycontrolDON is a rapid and precise quantitative method for analyzing Deoxynivalenol (DON). It has been specifically designed and calibrated for the analysis of food and feed and includes a sample preparation with solid phase extraction (SPE) columns. Samples in the µg/kg range (ppb = parts per billion range) can be analysed for DON in 6-10 minutes.

aokinmycontrolDON 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. aokinReferenceMatrixMaterials) 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	3 minutes
Time required for measurement	3 minutes
Analysis	
	Measurement range [µg/kg]
Range 1	50– 1250
Range 2	100– 2500
Range 3	200– 5000

### Deoxynivalenol

Deoxynivalenol (DON) is a mycotoxin produced by *Fusarium spec.* It is a suspected carcinogen, with acute poisoning resulting in vomiting.

National and international regulatory agencies have set permissible limits on the amount of DON allowed in food and feeds. As a consequence, it is strongly recommended to monitor DON content in all grain and corn food and feed raw materials and products.

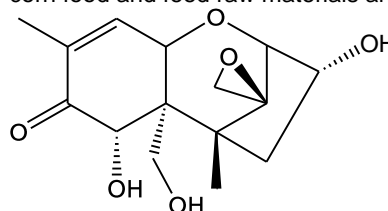


Figure 3: Chemical formula for deoxynivalenol  
 $C_{15}H_{20}O_6$ . Molecular weight: 296,3 g/mol

### Recommended Accessories

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

aokinextractor (food blender)	Order No.: 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 pipette (1000 µl)	LB-04-05-1000
Pipette tips (1000 µl)	LB-04-08-1000
Funnels	LB-05-04
Dispensette	LB-08-01
aokinReferenceMatrixMaterial	RMM-02

### 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**, 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 5 g of your sample, and 32 g extraction solution (32 ml *aokinExtractionSolventDON* at 20°C) directly into the extraction beaker. Preferentially the exact volume is applied using a dispensette.



Figure 4: Weighing

Close the extraction beaker with the lid (with the blending knives). Start mixing for 1 minute.



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

Alternatively, a magnetic stirrer can be used for a minimum of 10 minutes.

### 4. Direct use of extraction-supernatant

Wait a few seconds until enough supernatant appears and continue with step 4.

**Alternatively do a filtration:** Place the filter on a suitable funnel and the funnel onto a collection container. Open the extraction beaker, pour the extract onto the filter and collect the filtrate (at least 20 mL). Discard the filter paper and filter cake. Shake/stir the filtrate to ensure homogeneity.

### 5. Use of *aokinQuickClean* column

Place an *aokinQuickCleanDON* column in a collection tube and add 450 µl of the supernatant (or filtrate; Step 3). Place it in the centrifuge and spin for 2 minutes at 5000 x g.

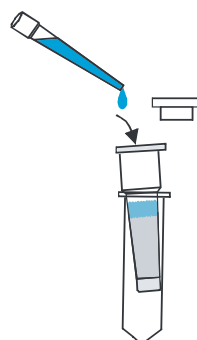


Figure 6: Pipetting of the extract onto the *aokinQuickCleanDON* column

### 5. Analyzing

Use the column filtrate for analyzing in the *aokinspectrometerFP470*.

Please follow detailed instructions for spectrometer use (*aokinspectrometerFP470* & *aokinLHW03* 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 DON*-glass container, filled with negative control *DON* solution = *aokinExtractionSolvent DON* 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.

### 6. 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 results remain high perform an offset correction of the calibration based on the negative control results. In addition, the use of recovery corrections preferentially by using sample extracts from certified reference matrix samples or alternatively based on the positive controls included in the kit.

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)

### Step 1: Extraction

- Sample mass:  $m_{\text{Sample}} = 5 \text{ g}$
- Volume extraction solvent:  $V_{\text{Extraction solvent}} = 32 \text{ mL}$
- Molar mass Deoxynivalenol:  $MW_{\text{DON}} = 296,3 \left[ \frac{\text{g}}{\text{mol}} \right]$

Mycotoxin concentration in the sample extract:

$$c \left[ \frac{\mu\text{mol}}{\text{l}} \right]_{\text{Extract}} = \frac{m_{\text{Sample}} [\text{kg}]}{V_{\text{Solvent}} [\text{l}] * MW_{\text{Mykotoxin}} \left[ \frac{\text{g}}{\text{mol}} \right]} * c \left[ \frac{\mu\text{g}}{\text{kg}} \right]_{\text{Sample}} = \frac{0.005}{0.032 * 296,3} * c \left[ \frac{\mu\text{g}}{\text{kg}} \right]_{\text{Sample}} = 0.0005273 * c \left[ \frac{\mu\text{g}}{\text{kg}} \right]_{\text{Sample}}$$

### Step 2: Purification

with **aokinQC****DON**

- Volume sample extract load to the **aokin** QC column:  $V_{\text{loaded sample extract}} = 0,45 \text{ ml}$
- Volume eluate from the **aokin** QC column:  $V_{\text{elute}} = 0,45 \text{ ml}$

Mycotoxin concentration in the column eluate:

$$c \left[ \frac{\mu\text{mol}}{\text{l}} \right]_{\text{Eluate}} = \frac{V_{\text{load}} [\text{ml}]}{V_{\text{elute}} [\text{ml}]} * c \left[ \frac{\mu\text{mol}}{\text{l}} \right]_{\text{Extract}} = \frac{0.45}{0.45} * c \left[ \frac{\mu\text{mol}}{\text{l}} \right]_{\text{Extract}} = 1 * c \left[ \frac{\mu\text{mol}}{\text{l}} \right]_{\text{Extract}}$$

### Step 3: Measurement

**aokin**FP 470 / LHW 03

- Sample volume:  $V_{\text{column eluate}} = V_{\text{sample}} = 200 \mu\text{l}$
- Total volume in the cuvette:  $V_{\text{cuvette}} = 2600 \mu\text{l}$

Mycotoxin concentration in the cuvette:

$$c \left[ \frac{\mu\text{mol}}{\text{l}} \right]_{\text{Cuvette}} = \frac{V_{\text{Sample}} [\mu\text{l}]}{V_{\text{cuvette}} [\mu\text{l}]} * c \left[ \frac{\mu\text{mol}}{\text{l}} \right]_{\text{Eluate}} = \frac{200}{2600} * c \left[ \frac{\mu\text{mol}}{\text{l}} \right]_{\text{Eluate}} = 0.0769 * c \left[ \frac{\mu\text{mol}}{\text{l}} \right]_{\text{Eluate}}$$

### Conversion factor: Extraction, Purification and Measurement

It follows the conversion factor from 1 to 3 above:

$$c \left[ \frac{\mu\text{mol}}{\text{l}} \right]_{\text{Cuvette}} = 0,0005273 * 1 * 0,0769 * c \left[ \frac{\mu\text{g}}{\text{kg}} \right]_{\text{Sample}} = 0,0000405 * c \left[ \frac{\mu\text{g}}{\text{kg}} \right]_{\text{Sample}} \quad \text{or}$$

$$c \left[ \frac{\mu\text{g}}{\text{kg}} \right]_{\text{Sample}} = \frac{1}{0,0405} * c \left[ \frac{\text{nmol}}{\text{l}} \right]_{\text{Cuvette}} = 24,691 * c \left[ \frac{\text{nmol}}{\text{l}} \right]_{\text{Cuvette}}$$

Dilution scheme of the standard solution sent for control purposes:

DON Cal 145,8



**Calibration:**

Dilution scheme (example for standard experimental setup of 200 µl sample into 2600 µl of total cuvette volume):

**DON Cal 167,7**

(→ **167.7 nM in cuvette**)

↓ **1:2 dilution** in *negative control DON*

(→ **83.85 nM in cuvette**)

↓ **1:2 dilution** in *negative control DON*

(→ **41.93 nM in cuvette**)

↓ **1:2 dilution** in *negative control DON*

(→ **20.96 nM in cuvette**)

*negative control DON*

(→ **0.00 nM in cuvette**)

**Positive control:**

Dilution scheme (example for standard experimental setup of 200 µl sample into 2600 µl of total cuvette volume):

**DON Cal 145,8**

200 µl in RANGE 1

(1895 nM in vial -----→ 145.8 nM in cuvette  
**in calculated volume for solid sample → 3600 µg/kg**)

100 µl in RANGE 2

(947,5 nM in vial -----→ 72,9 nM in cuvette  
**in calculated volume for solid sample → 1800 µg/kg**)

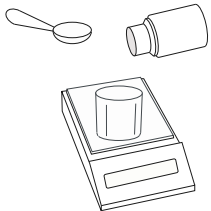
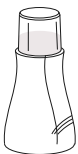

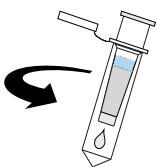
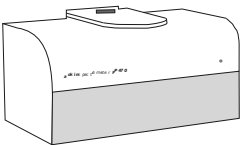
50 µl in RANGE 3

(473,8 nM in vial -----→ 36,5 nM in cuvette  
**in calculated volume for solid sample → 900 µg/kg**)

## Deoxynivalenol / standard samples:

- Recommended for **wheat and corn** flour samples
- Not suitable for bran products → please use the sample preparation for **absorbent samples** (*aokinmycontrolDON Absorbent*)
- Not suitable for barley and oat → please use the sample preparation for **special matrices** (*aokinmycontrolDON Special Matrix*)

 **Standard**

Extraction		<b>Weighing:</b>  5 g sample 32 mL <i>aokinExtractionSolventDON</i>
		<b>Extraction:</b>  1 min mixing
		<b>Filtration:</b>  collect filtrate (discard filter cake)
Purification		<b>SPE-Filtration:</b>  500 µl filtrate on <i>aokinQuickClean</i> column 2 min centrifuge at 5000 x g, use column filtrate for measurement
Measurement		<b>Automatic Analyse (FP470 / LHW03)</b>  place the 2ml-reaction tube in the sample holder of the <i>LHW03</i>  2200 µl <i>aokinReaction buffer</i> 200 µl sample (diluted 1:1 - RANGE 1) (diluted 1:2 - RANGE 2) (diluted 1:4 - RANGE 3)  100 µl <i>aokinmycontrolDON</i> Reagent 1 100 µl <i>aokinmycontrolDON</i> Reagent 2

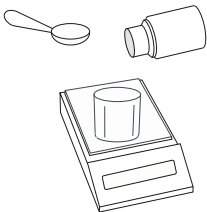
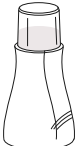

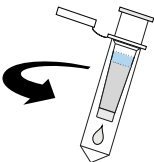
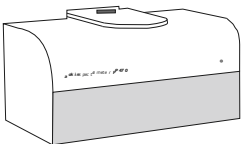
*DON = Deoxynivalenol, Conversion factor: 1 nmol DON/l in cuvette = 24,691 µg/kg*

## Deoxynivalenol / absorbent samples:

- Recommended for highly absorbent samples as **wheat or bran**
- Suitable for **all highly absorbent samples**
- Not suitable for standard samples → please use the sample preparation for **standard samples (aokinmycontrolDON Standard)**
- Not suitable for barley and oat → please use the sample preparation for **special matrices (aokinmycontrolDON Special Matrix)**

### Procedure:

**aokinmycontrolDON**  
**Absorbent**

Extraction		<b>Weighing:</b>  5 g sample 64 mL <b>aokinExtractionSolventDON</b>
		<b>Extraction:</b>  1 min mixing
		<b>Filtration:</b>  collect filtrate (discard filter cake)
Purification		<b>SPE-Filtration:</b>  500 µl filtrate on <b>aokinQuickClean</b> column 2 min centrifuge at 5000 x g, use column filtrate for measurement
Measurement		<b>Automatic Analyse (FP470 / LHW03)</b>  place the 2ml-reaction tube in the sample holder of the <b>LHW03</b>  2200 µl <b>aokinReaction buffer</b> 200 µl sample (diluted 1:1 - RANGE 1) (diluted 1:2 - RANGE 2) (diluted 1:4 - RANGE 3)  100 µl <b>aokinmycontrolDON Reagent 1</b> 100 µl <b>aokinmycontrolDON Reagent 2</b>

DON = Deoxynivalenol, Conversion factor: 1 nmol DON/l in cuvette = 49,382 µg/kg

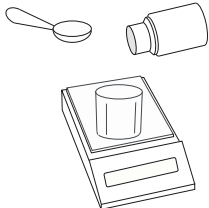
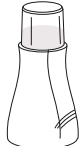

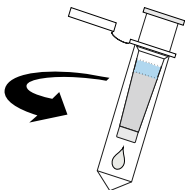
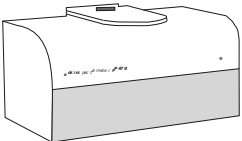


## Deoxynivalenol / special matrix:

- Recommended for **barley and oats**
- Suitable for all **non-highly absorbent samples**
- Not suitable for standard samples → please use the sample preparation for **standard samples (aokinmycontrolDON Standard)**
- Not suitable for bran products → please use the sample preparation for **absorbent samples (aokinmycontrolDON Absorbent)**

### Procedure:

**aokinmycontrolDON**  
**Special Matrix**

Extraction		<b>Weighing:</b>  5 g sample 32 mL <b>aokinExtractionSolventDON</b>
		<b>Extraction:</b>  1 min mixing
		<b>Filtration:</b>  collect filtrate (discard filter cake)
Purification		<b>SPE-Filtration:</b> <b>1. column</b> : 600 µl filtrate on <b>aokinQuickClean</b> column 2 min centrifuge at 5000 x g <b>2. column</b> filtrate of the 1 <sup>st</sup> <b>aokinQuickClean</b> on : 2 <sup>nd</sup> <b>aokinQuickClean</b> column ≥ 400 µl centrifuge at 5000 x g, use second column filtrate for measurement 2 min
Measurement		<b>Automatic Analyse (FP470 / LHW03)</b>  place the 2ml-reaction tube in the sample holder of the <i>LHW03</i> 2200 µl <b>aokinReaction buffer</b> 200 µl sample <i>(diluted 1:1 - RANGE 1)</i> <i>(diluted 1:2 - RANGE 2)</i> <i>(diluted 1:4 - RANGE 3)</i> 100 µl <b>aokinmycontrolDON Reagent 1</b> 100 µl <b>aokinmycontrolDON Reagent 2</b>

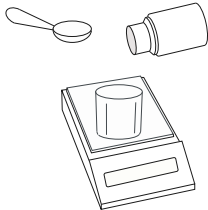
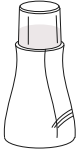

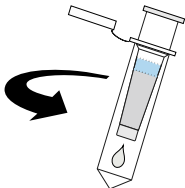
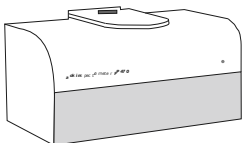
DON = Deoxynivalenol, Conversion factor: 1 nmol DON/l in cuvette = 24,691 µg/kg

## Deoxynivalenol / low contaminated standard samples:

- Recommended for **low contaminated samples**,  
limit of detection (LOD) 20 µg/kg
- Recommended standard samples like **wheat, triticale, corn and other**
- Suitable for all **non-highly absorbent samples**
- For highly absorbent, low loaded samples → please use the sample preparation for **low loaded absorbent samples (aokinmycontrolDON LOD 20 µg/kg, Absorbent)**

Procedure:

**aokinmycontrolDON**  
**LOD 20 µg/kg, Standard**

Extraction		<b>Weighing:</b>  15 g sample 32 mL <b>aokinExtractionSolventDON</b>
		<b>Extraction:</b>  1 min mixing
		<b>Filtration:</b>  collect filtrate (discard filter cake)
Purification		<b>SPE-Filtration:</b>  1. <b>column</b> : filtrate on <b>aokinQuickClean</b> column 600 µl centrifuge at 5000 x g 3 min  2. <b>column</b> : filtrate of the 1 <sup>st</sup> <b>aokinQuickClean</b> on 2 <sup>nd</sup> <b>aokinQuickClean</b> column ≥ 400 µl centrifuge at 5000 x g, use second column filtrate for measurement 3 min
Measurement		<b>Automatic Analyse (FP470 / LHW03)</b>  place the 2ml-reaction tube in the sample holder of the <b>LHW03</b>  2200 µl <b>aokinReaction buffer</b> 200 µl sample <i>(diluted 1:1 - RANGE 1)</i> <i>(diluted 1:2 - RANGE 2)</i> <i>(diluted 1:4 - RANGE 3)</i>  100 µl <b>aokinmycontrolDON Reagent 1</b> 100 µl <b>aokinmycontrolDON Reagent 2</b>

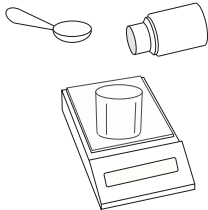
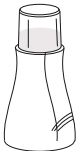

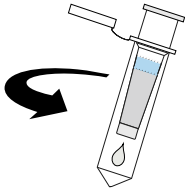
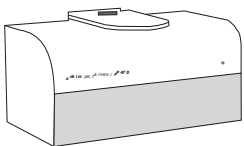
DON = Deoxynivalenol, Conversion factor: 1 nmol DON/ l in cuvette = 8,23 µg/kg

## Deoxynivalenol / low contaminated absorbent samples:

- Recommended for **low contaminated samples**,  
limit of detection (LOD) 20 µg/kg
- Recommended standard samples like **wheat, triticale, corn and other**
- Suitable for all **highly absorbent samples**
- For non highly absorbent, low loaded samples → please use the sample preparation for **low loaded standard samples (aokinmycontrolDON LOD 20 µg/kg, Standard)**

Procedure:

**aokinmycontrolDON**  
**LOD 20 µg/kg, Absorbent**

Extraction		<b>Weighing:</b>  10 g sample 32 mL <b>aokinExtractionSolventDON</b>
		<b>Extraction:</b>  1 min mixing
		<b>Filtration:</b>  collect filtrate (discard filter cake)
Purification		<b>SPE-Filtration:</b>  1. <b>column</b> : 900 µl filtrate on <b>aokinQuickClean</b> column centrifuge at 5000 x g 3 min  2. <b>column</b> : ≥ 650 µl filtrate of the 1 <sup>st</sup> <b>aokinQuickClean</b> on 2 <sup>nd</sup> <b>aokinQuickClean</b> column centrifuge at 5000 x g, use second column filtrate for measurement 3 min
Measurement		<b>Automatic Analyse (FP470 / LHW03)</b>  place the 2ml-reaction tube in the sample holder of the <b>LHW03</b>  2000 µl <b>aokinReaction buffer</b> 400 µl sample <i>(diluted 1:1 - RANGE 1)</i> <i>(diluted 1:2 - RANGE 2)</i> <i>(diluted 1:4 - RANGE 3)</i>  100 µl <b>aokinmycontrolDON Reagent 1</b> 100 µl <b>aokinmycontrolDON Reagent 2</b>

DON = Deoxynivalenol, Conversion factor: 1 nmol DON/l in cuvette = 6,163 µg/kg