INSTRUCTIONS FOR USE

aokin mycontrol DON

Order No.: MYS-QC-02

Quantitative determination of **Deoxynivalenol**

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

Package content:

Order No. MYS-QC-02
aokin*mycontrol* ***DON***

Order No	**Material**	**Content**	**Application**	**Storage**
MYS-QC-02-20 for 20 measurements	***aokin****mycontrol* ***DON*** Kit for quantitative determination of Deoxynivalenol (DON)	***aokin*** *EasyExtract* ***DON*** *Component A* ①, 1 pouch ***aokin*** *EasyExtract* ***DON*** *Component B* ②, 1 unit *sticker for* ***aokin*** *EasyExtractBuffer* ***DON*** ①+② ***aokin****QuickClean* ***DON*** ③, 20 units ***aokin*** *ReactionBuffer* ④, 1 bottle ***DON*** *Reagent* ⑤, 1 unit ***DON*** *Tracer* ⑤, 1 unit ***DON*** *positive control*, *Standard* ***DON*** ⑤, 1 unit	Measurement on ***aokin*** **FP analyzer**	Refrigerated Do not freeze
MYS-QC-02-100 for 100 measurements	***aokin****mycontrol* ***DON*** Kit for quantitative determination of Deoxynivalenol (DON)	***aokin*** *EasyExtract* ***DON*** *Component A* ①, 4 pouches ***aokin*** *EasyExtract* ***DON*** *Component B* ②, 4 units *sticker for* ***aokin*** *EasyExtractBuffer* ***DON*** ①+② ***aokin****QuickClean* ***DON*** ③, 100 units ***aokin*** *EasyReaction Buffer Component A* ④Ⓐ, 1 unit ***aokin*** *EasyReaction Buffer Component B* ④Ⓑ, 1 unit *sticker for* ***aokin*** *EasyReactionBuffer* ④ ***DON*** *Reagent* ⑤, 5 units ***DON*** *Tracer* ⑤, 5 units ***DON*** *positive control*, *Standard* ***DON*** ⑤, 5 units	Measurement on ***aokin*** **FP analyzer**	Refrigerated Do not freeze

Analytical-kit for rapid and quantitative determination of Deoxynivalenol (DON) in wheat, corn and other grains or feed.

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-02-20 for 20 measurements:
The complete content of the *EasyExtract* ***DON*** *Component A* ① pouch plus the *EasyExtract* ***DON*** *Component B* ② needs to be dissolved in a total of 1 L destilled or deionized water = ***aokin*** *EasyExtractBuffer* ***DON*** ①+②. Prepare the solution and label the container with the sticker ***aokin*** *EasyExtractBuffer* ***DON*** ①+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-02-100 for 100 measurements:
The complete content of the *EasyExtract* ***DON*** *Component A* ① pouch plus the *EasyExtract* ***DON*** *Component B* ② needs to be dissolved in a total of 1 L destilled or deionized water = ***aokin*** *EasyExtractBuffer* ***DON*** ①+②. Prepare 4x the solution and label the containers with stickers ***aokin*** *EasyExtractBuffer* ***DON*** ①+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:

aokin *EasyExtractBuffer* ***DON*** ①+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 (***DON*** *Reagent* ⑤, ***DON*** *Tracer* ⑤, *positive control* ⑤) must be stored in a refrigerator (2-10°C).

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 – 200 µL, 100-1000 µL and related pipette tips, 2 mL collection tubes, tube vortex mixer, centrifuge, timer, deionized water for buffer reconstitution.

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.

aokin**mycontrol* ***DON Special Matrix (sample preparation for barley and oats) and ***aokin**mycontrol* ***DON*** Sensitive (sample preparation for low contaminated standard samples like wheat, triticale and corn or low contaminated absorbent samples) require additional matrix removal columns (***aokin**QuickClean* ***DON*** ③, order number QC-02-100), which are not included in the regular ***aokin**mycontrol* ***DON*** Kit (MYS-QC-02).

Introduction

aokin**mycontrol* ***DON is a rapid method for quantifying Deoxynivalenol (DON). It has been specifically designed and calibrated for the analysis of food and feed and includes a sample preparation with matrix removal columns (***aokin**QuickClean* ***DON*** ③). Samples in the µg/kg range (µg/kg = ppb) can be analysed for DON in under 10 minutes.

aokin**mycontrol* ***DON is available with a calibration, which has been validated for grains and other food and feed products. 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 DON = ***aokin** EasyExtractBuffer* ***DON*** ①+②

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 DON ⑤ = Standards diluted in prepared ***aokin** EasyExtractBuffer* ***DON*** ①+②
Deoxynivalenol 100 ng/mL in Negative control DON, use 20 to 180 µL within the assay, fill up with Negative control DON (***aokin** EasyExtractBuffer* ***DON*** ①+②) for overall 200 µL sample.
(Example: 100 µL **Deoxynivalenol 100 ng/mL** + 100 µL Negative control DON (***aokin** EasyExtractBuffer* ***DON*** ①+②) = 50 ng/mL DON within the assay)

Deoxynivalenol

Deoxynivalenol (DON) is a mycotoxin produced by *Fusarium* molds. 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 feed. As a consequence, it is strongly recommended to monitor DON content in all grain, corn, food and feed raw materials and products.

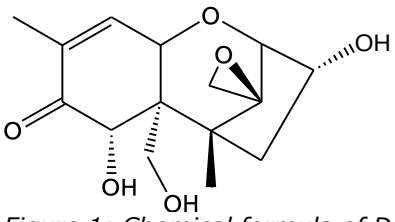


Figure 1: Chemical formula of Deoxynivalenol ($C_{15}H_{20}O_6$. Molecular weight: 296,3 g/mol)

Standard sample preparation for wheat and corn (Page 8/11)
(for other types of samples follow the instructions on the last pages)

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* ***DON*** ①+② = *EasyExtract* ***DON*** *Component A* ① + *EasyExtract* ***DON*** *Component B* ② dissolved in deionized water.
Negative control DON = ***aokin*** *EasyExtractBuffer* ***DON*** ①+②.
Positive control DON = DON Standard ⑤ diluted in Negative control DON.

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 5 g of your sample and add 35 mL ***aokin*** *EasyExtractBuffer* ***DON*** ①+② directly into an extraction beaker. Preferentially the exact volume is applied using a dispensette.
Extract sample with ***aokin*** *EasyExtractBuffer* ***DON*** by blending for 1 minute at high speed.
Alternatively, a magnetic stirrer can be used for a minimum of 10 minutes for extraction.

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 ***DON*** ③ column in a 2 mL collection tube and add 500 - 600 µL of the filtrate/supernatant (preferable 500 µL) (*Figure 2*). Place it in the centrifuge and spin for 2 minutes at 2000 - 6000 x g (preferable 5000 x g) or until all liquid passed the column. Your sample is now ready for analysis.

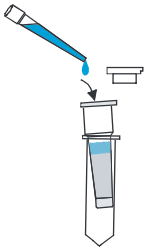


*Figure 2: Pipetting of the extract onto the **aokin**QuickClean column*

7. Introduction of general testing procedure

7.1. Pipette 860 µl of ***aokin*** *ReactionBuffer* ④ into the test tube.
7.2. Add 200 µL of clear sample extract (if necessary fill up with Negative control DON solution if sample extract is too concentrated, example: 100 µL sample + 100 µL Negative control DON for overall 200 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.

7.3. Add 20 µL of ***DON*** *Reagent (**BLUE** cap)* ⑤ into the test tube.
7.4. Mix well/vortex (without spilling).
7.5. 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.
7.6. Add 20 µL of ***DON*** *tracer (**RED** cap)* ⑤ into the first test tube.
7.7. Mix well.
7.8. Incubate for 60 seconds.
7.9. Obtain tracer measurement of the sample.
7.10. Repeat steps 7.6 to 7.9 for all test tubes.

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

8. Data acquisition and analysis

8.1. Mix and Read Process

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

8.2. 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.
$P = (\Delta H - \Delta V)/ (\Delta H + \Delta V)$.

8.3. 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

9. Daily Check I:

a. Use negative control (= *aokin* *EasyExtractBuffer* DON ①+②) in Step 7.2
b. Software: Choose *Daily Check I* in the user interface or alternatively *Offset at given T°C* Sheet in particular excel version of software

9.1. Pipette 860 µl of ***aokin*** *ReactionBuffer* ④ into a test tube.
9.2. Add 200 µL of a negative control into the test tube. The negative control for DON is the prepared ***aokin*** *EasyExtractBuffer* ***DON*** ①+②.
9.3. Add 20 µL of ***DON*** *Reagent* (***BLUE*** *cap)* ⑤ into the test tube.
9.4. Mix well/vortex (without spilling).
9.5. Obtain background measurement reading.
9.6. Add 20 µL of ***DON*** *Tracer* (***RED*** cap) ⑤ into the first test tube.
9.7. Mix well.
9.8. Incubate for 60 seconds.
9.9. Obtain tracer measurement.

Note: Run negative controls in triplicate.

10. Daily Check II-A:
a. Use liquid positive control in Step 7.2
b. Software: Choose *Daily Check II, Liquid standard* in the user interface or alternatively *Recovery rate at given T°C* Sheet in particular excel version of software

10.1. Pipette 860 µl of ***aokin****ReactionBuffer* ④ into a test tube.
10.2. Add 200 µL of a positive control into the test tube. The positive control is a liquid standard diluted in prepared ***aokin*** *EasyExtractBuffer* ***DON*** ①+②.
Respectively **Deoxynivalenol 100 ng/mL** in Negative control DON, use 20 to 180 µL within the assay, fill up with Negative control DON (***aokin*** *EasyExtractBuffer* ***DON*** ①+②) for overall 200 µL sample.
(Example: 100 µL **Deoxynivalenol 100 ng/mL** + 100 µL Negative control DON (***aokin*** *EasyExtractBuffer* ***DON*** ①+②) = 50 ng/mL DON within the assay)
10.3. Add 20 µL of ***DON*** *Reagent* (***BLUE*** *cap)* ⑤ into the test tube.
10.4. Mix well/vortex (without spilling).
10.5. Obtain background measurement reading.
10.6. Add 20 µL of ***DON*** *Tracer* (***RED*** cap) ⑤ into the first test tube.
10.7. Mix well.
10.8. Incubate for 60 seconds.
10.9. Obtain tracer measurement.

Note: Run positive controls in duplicate.

11. Alternative Daily Check II-B:
a. Use of a final extract of a reference matrix material as positive control in Step 7.2
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

11.1. 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.
11.2. Prepare the reference matrix sample according to Steps 4, 5, 6.
11.3. Pipette 860 µl of ***aokin*** *ReactionBuffer* ④ into a test tube.
11.4. Add 200 µL of the reference matrix extract into the test tube.
11.5. Add 20 µL of ***DON*** *Reagent* (***BLUE*** *cap)* ⑤ into the test tube.
11.6. Mix well/vortex (without spilling).
11.7. Obtain background measurement reading.
11.8. Add 20 µL of ***DON*** *Tracer* (***RED*** cap) ⑤ into the first test tube.
11.9. Mix well.
11.10. Incubate for 60 seconds.
11.11. Obtain tracer measurement.

Note: Run positive controls in duplicate. Repeat with a different dilution (Step 7.2), if needed.

12. Testing procedure – Sample:
a. Use sample extract in in Step 7.2
b. Software: Choose *Analysis* in the user interface or *Analysis at given T°C* Sheet in particular excel version of software

12.1. Pipette 860 µl of ***aokin*** *ReactionBuffer* ④ into the test tube.
12.2. Add 200 µL of clear sample extract or a control into the test tube.

12.3. Add 20 µL of ***DON*** *Reagent* (***BLUE*** *cap)* ⑤ into the test tube.
12.4. Mix well/vortex (without spilling).
12.5. Obtain background measurement readings of all samples and controls.
12.6. Add 20 µL of ***DON*** *Tracer* (***RED*** cap) ⑤ into the first test tube.
12.7. Mix well.
12.8. Incubate for 60 seconds.
12.9. Obtain tracer measurement of the first sample.
12.10. Repeat Steps 12.6 to 12.9 for all test tubes.

Note: Run samples as duplicates. Repeat with a different dilution (Step 7.2), if needed.

13. Test validation

13.1. The mean negative control must read between 160 and 230 mP.
13.2. The positive control must always read lower than the negative control. Expected values of the positive control are 10 mP to 50 mP lower than the negative control.
13.3. 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.
13.4. 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.

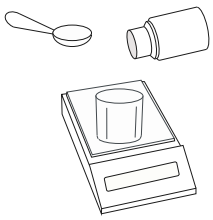
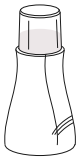
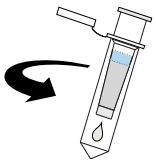
Note: Repeat the daily checks after each hour of testing, or on each microtiter plate. Performing measurements of controls regularly will ensure the accuracy of your determinations. The results of the daily checks are used to normalize the readings, this ensures the analytical performance.

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

Standard

Sample preparation for **wheat, wheat flour, titricale, corn, corn flour**
Deoxynivalenol LOD 35 µg/kg

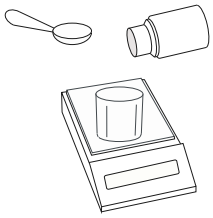
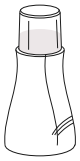
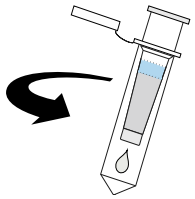
(Sample preparation for **bran and other absorbent samples** please use double amount of ***aokin*** *EasyExtractBuffer* ***DON***, *Deoxynivalenol LOD 70 µg/kg*)

Extraction		**Weighing:** 5 g sample 35 mL ***aokin*** *EasyExtractBuffer* ***DON*** ①+②
		**Extraction:** 1 min mixing
		**Filtration:** collect filtrate (discard filter cake)
Purification		**SPE-Filtration:** 500-600 µL filtrate on ***aokin****QuickClean* ***DON*** column ③ 2 min centrifuge at 2000 - 6000 x g or until all liquid passed column use filtrate for measurement
Measurement	Mix and read	**Quantification:** add into tube: 860 µL ***aokin*** *ReactionBuffer* ④ 200 µL sample 20 µL ***DON*** *Reagent* ***BLUE*** ⑤ mix well measure background 20 µL add ***DON*** *Tracer* ***RED*** ⑤ mix well, incubate 60 sec measure tracer

Special Matrix

Sample preparation for **barley and oats**

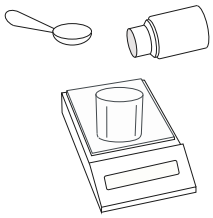
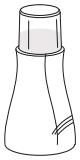
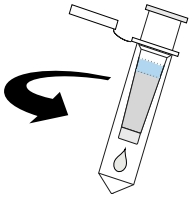
Deoxynivalenol LOD 35 µg/kg

Extraction		**Weighing:** 5 g sample 35 mL ***aokin** EasyExtractBuffer* **DON** ①+②
		**Extraction:** 1 min mixing
		**Filtration:** collect filtrate (discard filter cake)
Purification		**SPE-Filtration:** **1. column:** 600 µL filtrate on ***aokin**QuickClean* ***DON*** column ③ 2 min centrifuge at 2000 - 6000 x g **2. column:** ≥ 400 µL filtrate of the 1st ***aokin**QuickClean* on 2nd ***aokin**QuickClean* column ③ 2 min centrifuge at 2000 - 6000 x g or until all liquid passed column use filtrate for measurement
Measurement	Mix and read	**Quantification:** add into tube: ***aokin** ReactionBuffer* ④ 860 µL sample 200 µL ***DON** Reagent **BLUE*** ⑤ 20 µL mix well measure background add ***DON** Tracer **RED*** ⑤ 20 µL mix well, incubate 60 sec measure tracer

Sensitive Standard

Sample preparation for **low contaminated standard samples** like **wheat, triticale and corn**

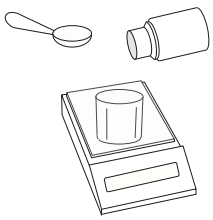
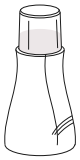
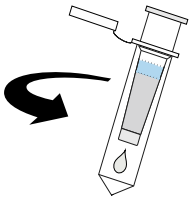
Deoxynivalenol LOD 12 µg/kg

Extraction		**Weighing:** 15 g sample 35 mL ***aokin*** *EasyExtractBuffer* **DON** ①+②
		**Extraction:** 1 min mixing
		**Filtration:** collect filtrate (discard filter cake)
Purification		**SPE-Filtration:** **1. column:** 600 µL filtrate on ***aokin****QuickClean* ***DON*** column ③ 2 min centrifuge at 2000 - 6000 x g **2. column:** ≥ 400 µL filtrate of the 1st ***aokin****QuickClean* on 2nd ***aokin****QuickClean* column ③ 2 min centrifuge at 2000 - 6000 x g or until all liquid passed column use filtrate for measurement
Measurement	Mix and read	**Quantification** add into tube: 860 µL ***aokin*** *ReactionBuffer* ④ 200 µL sample 20 µL ***DON*** *Reagent* ***BLUE*** ⑤ mix well measure background 20 µL add ***DON*** *Tracer* ***RED*** ⑤ mix well, incubate 60 sec measure tracer

Sensitive Absorbent

Sample preparation for **low contaminated absorbent samples**

Deoxynivalenol LOD 18 µg/kg

Extraction		**Weighing:** 10 g sample 35 mL ***aokin** EasyExtractBuffer* **DON** ①+②
		**Extraction:** 1 min mixing
		**Filtration:** collect filtrate (discard filter cake)
Purification		**SPE-Filtration:** **1. column:** 900 µL filtrate on ***aokin**QuickClean* ***DON*** column ③ 2 min centrifuge at 2000 - 6000 x g **2. column:** ≥ 600 µL filtrate of the 1st ***aokin**QuickClean* on 2nd ***aokin**QuickClean* column ③ 2 min centrifuge at 2000 - 6000 x g or until all liquid passed column use filtrate for measurement
Measurement	Mix and read	**Quantification** add into tube: 660 µL ***aokin** ReactionBuffer* ④ 400 µL sample 20 µL ***DON** Reagent **BLUE*** ⑤ mix well measure background 20 µL add ***DON** Tracer **RED*** ⑤ mix well, incubate 60 sec measure tracer