INSTRUCTIONS FOR USE

aokin mycontrol T2/HT2

Order No.: MY-QC-78

Sample preparation with aokinQuickClean columns (SPE)

aokin *mycontrol* **T2/HT2**

Analytical-kit for rapid and quantitative determina-tion of T-2- and HT-2-Toxin (T2/HT2).

Materials

aokinmycontrolT2/HT2 (Order No.: MY-QC-78-100)

Package content

A) *Materials for sample preparation:*
aokinExtractionSolventT2/HT2, Extraction solution
aokinExtractionSalt T2/HT2 + spoon
aokinQuickClean T2/HT2, centrifuge columns
aokinmycontrol T2/HT2 Precipitation buffer
(transparent cap)
Filter paper
Reaction tubes 2 mL

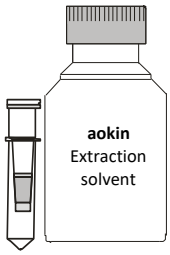


Figure 1: aokinQuickClean *column with reaction tube and* Extraction solvent *(1 L bottle)*

B) *Materials for analytical measurement:*
aokinReactionBuffer, Reaction buffer
aokinmycontrol T2/HT2, Reagent 1 (yellow cap),
F-T2/HT2, (for 5 analyses each)
aokinmycontrol T2/HT2, Reagent 2 (black cap),
A-T2/HT2, (for 5 analyses each)

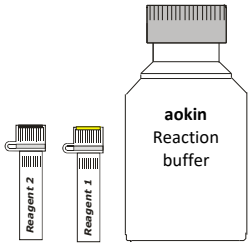


Figure 2:
Reagent 1, F-T2/HT2 (yellow cap), Reagent 2, A-T2/HT2 (black cap) and Reaction buffer (1 L bottle)

C) *Materials for internal quality control:*
aokin*mycontrol* T2/HT2, negative control T2/HT2
(transparent), for zero value measurements
aokinmycontrol T2/HT2, Reagent 1 (yellow cap),
F-T2/HT2, (for 5 analyses each)
aokinmycontrol T2/HT2, Reagent 2 (black cap),
A-T2/HT2, (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:
aokinmycontrol T2/HT2 (Order No.: MY-QC-78-100)

Introduction

aokinmycontrolT2/HT2 is a rapid and precise quantitative method for analyzing of T-2 and HT-2-Toxin (T2/HT2). It has been specifically designed and calibrated for the analysis of wheat 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 T-2 and HT-2-Toxin in 15 minutes.
aokinmycontrolT2/HT2 is available with a calibration, which has been validated for wheat. 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	wheat
Time required for sample preparation	12 minutes
Time required for measurement	3 minutes
Analysis	
	Measurement range [µg/kg]
Range 1	70 – 420
Range 2	140 – 840
Range 3	280 – 1680

T-2 and HT-2 Toxins

T-2 and HT-2 are mycotoxins. They naturally occur in molds by *Fusarium sp*. fungus. It is toxic to humans and animals. As a consequence, it is strongly recommended to monitor the content in grain and corn food and feed raw materials and products.

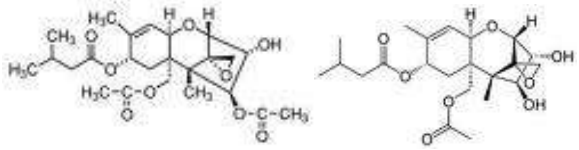


Figure 3: Chemical formula for T-2 Toxin ($C_{24}H_{34}O_9$ molecular weight: 466,52 g/mol) and HT-2 Toxin ($C_{22}H_{32}O_8$; molecular weight: 424,48 g/mol)

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-78

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* T2/HT2 and 30,4 g extraction solution (35 ml aokinExtractionSolvent T2/HT2 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 extraction protocol.

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

3. 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

4. Use of aokinQuickClean **column**

Place an aokinQuickClean **T2/HT2** column in a collection tube and add 900 µl of the filtrate (Figure 7). Place it in the centrifuge and spin for 3 minutes at 5.000 x g.Place second aokinQuickClean **T2/HT2** column in a collection tube and add ≥ 650 µL of the purified filtrate. Place it in the centrifuge and spin for 3 minutes at 5000 x g .

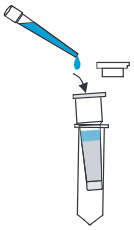


Figure 7: Pipetting of the extract onto the aokinQuickCleanT2/HT2 *column*

5. Precipitation

Add 400 µl of column-filtrate into the aokinmycontrol T2/HT2 precipitation buffer (trans-parent cap) and mix it well. In case a precipitation is visible centrifuge with maximum g-force (> 10.000 x g) for 5 minutes.

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

6. 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 T2/HT2*-glass container, filled with negative control T2/HT2 solution = 4 mL aokinExtraction Solvent T2/HT2 + 8 mL aokinmycontrol T2/HT2 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 Reaction buffer and check if tubing is below the surface.
6) Place a new cuvette with a clean stirrer into the spectrometer.

7. 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:* $m_{Sample} = 15$ g
- *Volume extraction solvent:* $V_{Extraction\ solvent} = 35$ ml
- *Molar mass T2:* $MW_{T2} = 445{,}555 \left[\frac{g}{mol}\right]$

Mycotoxin concentration in the sample extract:

$$\boldsymbol{c}\left[\frac{\mu\boldsymbol{mol}}{\boldsymbol{l}}\right]_{\boldsymbol{Extract}} = \frac{m_{Sample}[kg]}{V_{Solvent}[l] * MW_{Mykotoxin}\left[\frac{g}{mol}\right]} * c\left[\frac{\mu g}{kg}\right]_{Sample} = \frac{0.015}{0.035 * 445{,}555} * c\left[\frac{\mu g}{kg}\right]_{Sample} = 0.00096188 * c\left[\frac{\mu g}{kg}\right]_{Sample}$$

Step 2: Purification

with ***aokin*** *QC T2/HT2*

- *Volume sample extract load to the **aokin** QC column:* $V_{loaded\ sample\ extract} = 0{,}9$ ml
- *Volume eluate from the **aokin** QC column:* $V_{elute} = 0{,}9$ ml

Mycotoxin concentration in the column eluate:

$$\boldsymbol{c}\left[\frac{\mu\boldsymbol{mol}}{\boldsymbol{l}}\right]_{\boldsymbol{Eluate}} = \frac{V_{load\,[ml]}}{V_{elute\,[ml]}} * c\left[\frac{\mu mol}{l}\right]_{Extract} = \frac{0.9}{0.9} * c\left[\frac{\mu mol}{l}\right]_{Extract} = 1 * c\left[\frac{\mu mol}{l}\right]_{Extract}$$

Step 3: Dilution

- *Volume Eluate:* $V_{eluate} = 0{,}4$ ml
- *Total volume:* $V_{total} = 1{,}2$ ml

$$\boldsymbol{c}\left[\frac{\mu\boldsymbol{mol}}{\boldsymbol{l}}\right]_{\boldsymbol{Diluted}} = \frac{V_{eluate\,[ml]}}{V_{total\,[ml]}} * c\left[\frac{\mu mol}{l}\right]_{Extract} = \frac{0.4}{1.2} * c\left[\frac{\mu mol}{l}\right]_{Extract} = 0{,}333 * c\left[\frac{\mu mol}{l}\right]_{Extract}$$

Step 4: Measurement

aokin FP 470 / *LHW 03*

- *Sample volume:* $V_{Column\ eluate} = V_{Sample} = 600\ \mu l$
- *Total volume in the cuvette:* $V_{Cuvette} = 2600\ \mu l$

Mycotoxin concentration in the cuvette:

$$\boldsymbol{c}\left[\frac{\mu\boldsymbol{mol}}{\boldsymbol{l}}\right]_{\boldsymbol{Cuvette}} = \frac{V_{Sample\,[\mu l]}}{V_{cuvette\,[\mu l]}} * c\left[\frac{\mu mol}{l}\right]_{Eluate} = \frac{600}{2600} * c\left[\frac{\mu mol}{l}\right]_{Eluate} = 0.23 * \left[\frac{\mu mol}{l}\right]_{Eluate}$$

Conversion factor: Extraction, Purification and Measurement

It follows from 1 to 4 above the conversion factor

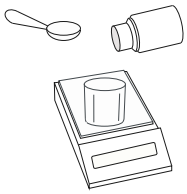
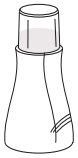
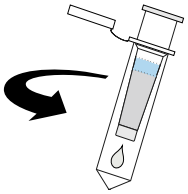
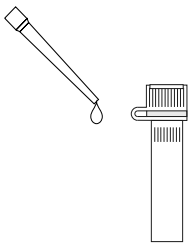
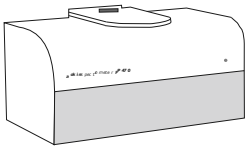
$$\boldsymbol{c}\left[\frac{\mu\,\boldsymbol{mol}}{\boldsymbol{l}}\right]_{\boldsymbol{Cuvette}} = 0{,}00096188 * 1 * 0{,}333 * 0.23 * c\left[\frac{\mu g}{kg}\right]_{Sample} = 0.00007367 * c\left[\frac{\mu g}{kg}\right]_{Sample} \quad \text{or}$$

$$\boldsymbol{c}\left[\frac{\mu\,\boldsymbol{g}}{\boldsymbol{kg}}\right]_{\boldsymbol{Sample}} = \frac{1}{0.07367} * c\left[\frac{nmol}{l}\right]_{Cuvette} = 13.515 * \boldsymbol{c}\left[\frac{\boldsymbol{nmol}}{\boldsymbol{l}}\right]_{\boldsymbol{Cuvette}}$$

T-2/HT-2 toxin / for wheat flour samples

***aokin**mycontrol T2/HT2*

Procedure: ***Standard***

Extraction		**Weighing:** 15 g sample 1,5 g ***aokin**ExtractionSalt **T2/HT2*** 35 mL ***aokin**ExtractionSolvent **T2/HT2***
		**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* column 3 min centrifuge at 5000 x g **2. column:** ≥ 650 µL filtrate of the 1st ***aokin**QuickClean* on 2nd ***aokin**QuickClean* column 3 min centrifuge at 5000 x g, use second column filtrate for measurement
Precipitation		**Precipitation:** 400 µl column filtrate into *Precipitation buffer* (transparent cap) 5 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* 1800 µL ***aokin** Reaction buffer* 600 µL sample *(diluted 1:1 - RANGE 1)* *(diluted 1:2 - RANGE 2)* *(diluted 1:4 - RANGE 3)* 100 µL ***aokin**mycontrol **T2/HT2*** Reagent 1

	100 µL ***aokin****mycontrol* ***T2/HT2*** Reagent 2

T2/HT2 = T2/HT2 Toxins, Conversion factor: 1 nmol T2/HT2/l in cuvette = 13,515 µg/kg in sample