INSTRUCTIONS FOR USE

mycontrol T2/HT2

Sample preparation with *QuickClean* columns (SPE)

mycontrol ***T2/HT2***

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

Materials

*mycontrol****T2/HT2***

Package content

A) *Materials for sample preparation:*

*ExtractionSolvent****T2/HT2,*** Extraction solution
ExtractionSalt ***T2/HT2*** + spoon
QuickClean ***T2/HT2,*** centrifuge columns
mycontrol ***T2/HT2*** Precipitation buffer (transparent cap)
Filter paper
Reaction tubes 2 mL

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

B) *Materials for analytical measurement:*

ReactionBuffer, Reaction buffer
mycontrol ***T2/HT2,*** Reagent 1 (yellow cap), F-T2/HT2, (for 5 analyses each)
mycontrol ***T2/HT2,*** Reagent 2 (black cap), A-T2/HT2, (for 5 analyses each)

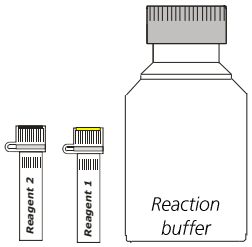


Figure 2: Reagent 1, F-T2/HT2 (yellow cap), Reagent 2, A-T2/HT2 (black cap) and Reaction buffer (1 L bottle)

C) *Materials for internal quality control:*

mycontrol ***T2/HT2,*** negative control T2/HT2 (transparent), for zero value measurements
mycontrol ***T2/HT2,*** Assay Additive (green cap), ADD-T2/HT2, (for 5 analyses each)
mycontrol ***T2/HT2,*** Reagent 1 (yellow cap), F-T2/HT2, (for 5 analyses each)
mycontrol ***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 +4°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:
aokin*mycontrol* ***T2/HT2***

Introduction

aokin*mycontrol****T2/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 13 minutes.
aokin*mycontrol****T2/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. ***aokin****ReferenceMatrix 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	10 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.

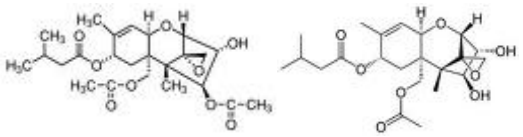


Figure 3: Chemical formula for T-2 Toxin ($C_{24}H_{34}O_9$ molecular weight: 466,52 g/mol) and HT-2 Toxin ($C_{22}H_{32}O_8$; molecular weight: 424,48 g/mol)

Recommended Accessories

All required materials are available

extractor (food blender)
watchbox (timer for food blender)
Weighing scale, d = 0,01 g
Eppendorf centrifuge, variable g-force
Variable pipettes (1000 µl)
Pipette tips (1000 µl)
Funnel
Dispensette
ReferenceMatrixMaterial

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. 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 *extractor.*

2. Weighing and extraction

Weigh 15 g of your sample, add one spoon (1,5 g) of *ExtractionSalt* ***T2/HT2*** and 30,4 g extraction solution (35 ml *ExtractionSolvent* ***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 *watchbox* (a preprogrammed timer) to conveniently and automatically complete this extraction protocol.

Figure 5: Extracting with the extractor (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 *QuickClean* column

Place an *QuickClean* ***T2/HT2*** 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 5.000 x g.

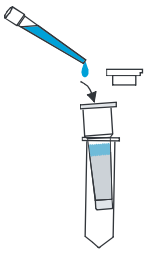


Figure 7: Pipetting of the extract onto the ***aokin****QuickClean****T2/HT2*** *column*

5. Precipitation

Add 100 µl of column-filtrate into the *mycontrol* ***T2/HT2*** precipitation buffer (transparent cap). In case a precipitation is visible centrifuge with maximum g-force (> 10.000 x g) for 5 minutes.

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

6. Analyzing

Please follow detailed instructions for spectrometer use.

This includes:

1) Place ***Reagents 1*** and ***2*** into position A6 and B6 of the sample rack of your spectrometer.
2) Fill up the ***Clean1*** solution and place a clean 2 mL vial in position A1.
3) Place an empty waste bottle in the holder. Check presence of ***Reaction buffer*** and check if tubing is below the surface.
4) Place a new cuvette with a clean stirrer into the spectrometer.

7. Quality control

There are free materials included in the kit, for your internal quality control: ***Reagent 1***, ***Reagent 2***, as well as ***negative control*** solutions for measurements of zero values (corresponding to samples free of mycotoxin).

Please regularly carry zero value measurements to ensure the accuracy of your measurements.

If you should measure increased zero values, please contact

Conversion factor: analyte concentration in cuvette (nM) to amount in sample (µg/kg)

mycontrol T2/HT2
Standard

Step 1: Extraction

- *Sample mass:* m_{Sample} = 15 g
- *Volume extraction solvent:* $V_{Extraction\ solvent}$ = 35 ml
- *Molar mass T2:* $MW_{T2} = 466{,}52\left[\frac{g}{mol}\right]$

Mycotoxin concentration in the sample extract:

$$\boldsymbol{c}\left[\frac{\boldsymbol{\mu mol}}{\boldsymbol{l}}\right]_{\boldsymbol{Extract}} = \frac{m_{Sample}[kg]}{V_{Solvent}[l] * MW_{Mykotoxin}\left[\frac{g}{mol}\right]} * c\left[\frac{\mu g}{kg}\right]_{Sample} = \frac{0.015}{0.035 * 466{,}52} * c\left[\frac{\mu g}{kg}\right]_{Sample} = 0.00091866 * c\left[\frac{\mu g}{kg}\right]_{Sample}$$

Step 2: Purification

with *QC T2/HT2*

- *Volume sample extract load to the QC column:* $V_{loaded\ sample\ extract}$ = 0,4 ml
- *Volume eluate from the 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} = 0,1 ml
- *Total volume:* V_{total} = 1 ml

$$\boldsymbol{c}\left[\frac{\boldsymbol{\mu mol}}{\boldsymbol{l}}\right]_{\boldsymbol{Diluted}} = \frac{V_{eluate\,[ml]}}{V_{total\,[ml]}} * c\left[\frac{\mu mol}{l}\right]_{Extract} = \frac{0.1}{1.0} * c\left[\frac{\mu mol}{l}\right]_{Extract} = 0{,}1 * c\left[\frac{\mu mol}{l}\right]_{Extract}$$

Step 4: Measurement

- *Sample volume:* $V_{Column\ eluate} = V_{Sample} = 400\ \mu l$
- *Total volume in the cuvette:* $V_{Cuvette} = 2600\ \mu l$

Mycotoxin concentration in the cuvette:

$$\boldsymbol{c}\left[\frac{\boldsymbol{\mu mol}}{\boldsymbol{l}}\right]_{\boldsymbol{Cuvette}} = \frac{V_{Sample}\,[\mu l]}{V_{cuvette}\,[\mu l]} * c\left[\frac{\mu mol}{l}\right]_{Eluate} = \frac{400}{2600} * c\left[\frac{\mu mol}{l}\right]_{Eluate} = 0.154 * \left[\frac{\mu mol}{l}\right]_{Eluate}$$

Conversion factor: Extraction, Purification and Measurement

It follows from 1 to 4 above the conversion factor

$$\boldsymbol{c}\left[\frac{\mu\,\boldsymbol{mol}}{\boldsymbol{l}}\right]_{\boldsymbol{Cuvette}} = 0{,}00091866 * 1 * 0{,}1 * 0.154 * c\left[\frac{\mu g}{kg}\right]_{Sample} = 0.00001415 * c\left[\frac{\mu g}{kg}\right]_{Sample} \quad \text{or}$$

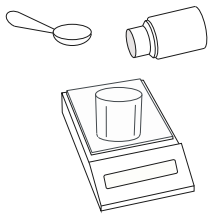
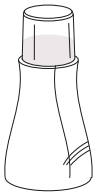
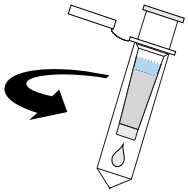
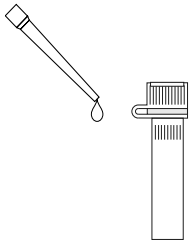
$$\boldsymbol{c}\left[\frac{\mu\,\boldsymbol{g}}{\boldsymbol{kg}}\right]_{\boldsymbol{Sample}} = \frac{1}{0.01415} * c\left[\frac{nmol}{l}\right]_{Cuvette} = \boldsymbol{70.67} * \boldsymbol{c}\left[\frac{\boldsymbol{nmol}}{\boldsymbol{l}}\right]_{\boldsymbol{Cuvette}}$$

T2/HT2 Toxin / standard samples:

- Recommended for wheat

Procedure:

mycontrol T2/HT2
Standard

Extraction		**Weighing:** 15 g sample 1,5 g *ExtractionSalt T2/HT2* 35 mL *ExtractionSolvent T2/HT2*
		**Extraction:** 3,5 min mixing with *watchbox*
		**Filtration:** collect filtrate (discard filter cake)
Purification		**SPE-Filtration:** 400 µL filtrate on *QuickClean* column 3 min centrifuge at 5.000 x g
Precipitation		**Precipitation:** 100 µl column filtrate into *Precipitation buffer* (transparent cap) 5 min centrifuge at > 10.000 x g transfer supernatant into clean 2 mL reaction tube