INSTRUCTIONS FOR USE

aokin mycontrol T2/HT2

Order No.: MYS-QC-78

Quantitative determination of the sum of **T-2 and HT-2 Toxins**

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

Package content:

Order No. MYS-QC-78 ***aokin****mycontrol* ***T2/HT2***
Kit for quantitative determination of T-2- nd HT-2 Toxins (T2/HT2)

Order No	**Content**	**Used with**	**Storage**
MYS-QC-78-20 for 20 measurements	***aokin****ExtractionSalt* ***T2/HT2,*** ① ***aokin*** *Easy Extract Liquid* ***T2/HT2*** ②, 1 vial *label for* ***aokin*** *EasyExtractBuffer* ***T2/HT2*** ② ***aokin****QuickClean* ***T2/HT2*** ③, 40 units ***aokin***Easy*PrecipitationBuffer****T2/HT2*** Ⓟ, 1 unit *label for* ***aokin*** *Precipitation buffer* ***T2/HT2*** Ⓟ ***aokin*** *ReactionBuffer* ④, 1 bottle ***T2/HT2*** *Reagent* ⑤, 1 unit ***T2/HT2*** *Tracer* ⑤, 1 unit ***T2/HT2*** *positive control, Standard* ***T2/HT2*** ⑤, 8 ng/mL T2 in **negative** ***T2/HT2***, 1 unit	***aokin*** **FP analyzer**	Refrigerated Do not freeze
MYS-QC-78-100 for 100 measurements	***aokin****ExtractionSalt* ***T2/HT2,*** ① ***aokin*** *Easy Extract Liquid* ***T2/HT2*** ②, 1 unit *label for* ***aokin*** *EasyExtractBuffer* ***T2/HT2*** ② ***aokin****QuickClean* ***T2/HT2*** ③, 200 units ***aokin***Easy*PrecipitationBuffer****T2/HT2*** Ⓟ, 1 unit *label for* ***aokin*** *Precipitation buffer* ***T2/HT2*** ***aokin*** *EasyReaction Buffer Component A* ④Ⓐ, 1 unit ***aokin*** *EasyReaction Buffer Component B* ④Ⓑ, 1 unit *label for* ***aokin*** *EasyReactionBuffer* ④ ***T2/HT2*** *Reagent* ⑤, 5 units ***T2/HT2*** *Tracer* ⑤, 5 units ***T2/HT2*** *positive control, Standard* ***T2/HT2*** ⑤, 8 ng/mL T2 in **negative** ***T2/HT2***, 5 units	***aokin*** **FP analyzer**	Refrigerated Do not freeze

Analytical-kit for rapid and quantitative determination of T-2 and HT-2 Toxins (T2/HT2) in wheat, oats, barley, corn and other grains.

Materials

All materials provided are precisely weighed. Control of the volumes used 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 is not advised.

Preparation of buffers:

MYS-QC-78-20 for 20 measurements:
1. Mix 190 mL deionized water with 10 mL of ***aokin*** *Easy Extract Liquid* ***T2/HT2*** ② and 800 mL of Methanol (analytical grade). => ***aokin*** *EasyExtractBuffer* ***T2/HT2*** ②. Label the container with the label provided in the kit (***aokin*** *EasyExtractBuffer* ***T2/HT2*** ②).
2. The ***aokin*** Easy*PrecipitationBuffer* ***T2/HT2*** *tablet* included in the package needs to be dissolved in 100 mL deionized water => ***aokin*** *EasyPrecipitationBuffer* ***T2/HT2*** Ⓟ.
3. Allow ***aokin*** *ReactionBuffer* ④ to adjust to room temperature (18-26°C) before using.

MYS-IC-78-100 for 100 measurements:
1. Mix 190 mL destilled or deionized water with 10 mL of ***aokin*** *Easy Extract Liquid* ***T2/HT2*** ② and 800 mL of Methanol (analytical grade). => ***aokin*** *EasyExtractBuffer* ***T2/HT2*** ②. Label the container with one of the labels provided in the kit (***aokin*** *EasyExtractBuffer* ***T2/HT2*** ②). There is enough material for 4 L.
2. Dissolve a tablet ***aokin*** *Precipitation buffer in 300 mL deionized water* => ***aokin*** *EasyPrecipitationBuffer* ***T2/HT2*** Ⓟ.
3. Dissolve the content of the *EasyReaction Buffer Component A* ④Ⓐ and *EasyReaction Buffer Component B* ④Ⓑ pouch 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 label ***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:

The prepared ***aokin*** *EasyExtractBuffer* ***T2/HT2*** ② is stable for 12 months without refrigeration (15° - 30°C).

aokin *ReactionBuffer* ④ is stable for 12 months without refrigeration (15° - 30°C).

The prepared ***aokin*** *EasyPrecipitationBuffer* ***T2/HT2*** Ⓟ is stable for 1 month with refrigeration (2° - 10°C).

Kit reagents (***T2/HT2*** *Reagent* ⑤, ***T2/HT2*** *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 – 100 µL, 100-1000 µL and related pipette tips, 2 mL collection tubes, tube vortex mixer, centrifuge, timer, deionized water for buffer reconstitution, methanol.

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.

Introduction

aokin*mycontrol* ***T2/HT2*** is a rapid method for quantifying T2/HT2 toxins (T2/HT2). It has been specifically designed and calibrated for the analysis of food. The kit process includes a sample preparation step with matrix removal columns (***aokin****QuickClean* ***T2/HT2***). Samples in the µg/kg range (µg/kg = ppb) can be analysed for T2/HT2 in under 15 minutes.

aokin*mycontrol* ***T2/HT2*** is available with a calibration, which has been validated for grains. Daily checks of negative control and positive control (either liquid standard or reference matrix extract) are required to ensure reliable results.

FPIA 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. Avoid temperature variations of more than 1°C during one determination.

Negative control T2/HT2 = 4 mL of prepared ***aokin*** *EasyExtractBuffer* ***T2/HT2*** ② diluted with 8 mL of ***aokin*** *EasyPrecipitationBuffer* **T2/HT2** Ⓟ

Positive controls:

Positive controls are required 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 T2/HT2 ⑤ = Standards diluted in *Negative control T2/HT2*

Positive control T2/HT2 ⑤ (8 ng T2/mL) *can be used in the range of* 40 to 220 µL directly in the assay by effectively diluting it with Negative control T2/HT2 *to a total of* 300 µL sample volume.

(Example: 100 µL **T2-Toxin 8 ng/mL** + 200 µL Negative control T2/HT2 => calculated concentration 2,67 ng/mL T2-Toxin)

T-2 and HT-2 Toxins

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

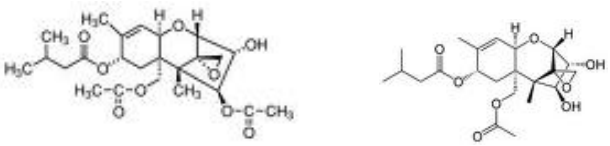


Figure 1: Chemical formula for T-2 Toxin ($C_{24}H_{34}O_9$ molecular weight: 466,52 g/mol) and HT-2 Toxin ($C_{22}H_{32}O_8$; molecular weight: 424,48 g/mol)

Standard sample preparation (see also page 8)

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* ***T2/HT2*** ② (= ***aokin*** *Easy Extract Liquid* ***T2/HT2*** ② dissolved in deionized water and Methanol).
aokin *EasyPrecipitationBuffer* ***T2/HT2*** Ⓟ: (= ***aokin*** *EasyPrecipitationBuffer****T2/HT2*** Ⓟ tablet dissolved in deionized water).
Negative control T2/HT2 *(*= prepared ***aokin*** *EasyExtractBuffer* ***T2/HT2*** ② diluted with ***aokin*** *EasyPrecipitationBuffer****T2/HT2*** Ⓟ).
Positive control T2/HT2 = Standard ⑤ diluted in Negative Control.

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 15 g of your sample, add one spoon (1.5 g) of ***aokin****ExtractionSalt* ***T2/HT2*** and 35 mL ***aokin*** *EasyExtractBuffer* ***T2/HT2*** directly into an extraction beaker. Preferentially the exact volume is applied using a dispensette.

Extract sample by blending for 2 minutes at high speed.

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* ***T2/HT2*** ③ column in a 2 mL collection tube and add 900 µL of the supernatant (or filtrate; Step 5). Place it in the centrifuge and spin for 3 minutes at 5000 x g. Place second ***aokin****QuickClean* ***T2/HT2*** ③ column in a 2 mL collection tube and add ≥ 650 µL of the purified filtrate from the first column. Place it again in the centrifuge and spin for 3 minutes at 5000 x g or until all liquid passed the column.

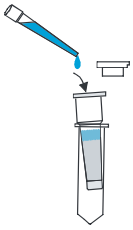


Figure 2: Pipetting of the extract onto the ***aokin****QuickClean column*

7. Precipitation

Add 400 µL of the column filtrate into 800 µL of the prepared ***aokin*** *Precipitation buffer* ***T2/HT2*** and mix it well. In case a precipitation is visible centrifuge with maximum g-force (> 10000 x g) for 5 minutes. Transfer the supernatant into a clean tube. Your sample is now ready for analysis.

8. Introduction of general testing procedure

8.1. Pipette 765 µl of ***aokin****ReactionBuffer* ④ into the test tube.
8.2. Add 300 µL of clear sample extract (if high in analyte than diluted with Negative control T2/HT2, example: 150 µl sample + 150 µl Negative control T2/HT2 for a total sample volume of 300 µl) or a control into the test tube.

Note: Run negative controls in triplicate, and positive control and samples as duplicates for best performance.

8.3. Add 15 µL of ***T2/HT2*** *Reagent (****BLACK*** *cap)* ⑤ into the test tube.
8.4. Mix well/vortex (without spilling). Incubate for 5 minutes.
8.5. Obtain background measurement.
8.6. Add 20 µL of ***T2/HT2*** *tracer (****YELLOW*** cap) ⑤ into the first test tube.
8.7. Mix well.
8.8. Incubate for 60 seconds.
8.9. Obtain tracer measurement of the sample.
8.10. Repeat steps 8.6 to 8.9 for all test tubes.

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

9. Data acquisition and analysis

9.1. Mix and Read Process

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

9.2. 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.
$P = (\Delta H - \Delta V)/ (\Delta H + \Delta V)$.

9.3. *aokin mycontrol* software

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 analyzer
- 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

10. Daily Check I:

a. Use negative control (= 4 mL of prepared *aokin* *EasyExtractBuffer* *T2/HT2* ② diluted with 8 mL of *aokin* *EasyPrecipitationBuffer* *T2/HT2* Ⓟ) in Step 8.2

b. Software: Choose *Daily Check I* in the user interface or alternatively *Offset at given T°C* Sheet in particular excel version of software

10.1. Pipette 765 µl of ***aokin****ReactionBuffer* ④ into a test tube.
10.2. Add 300 µL of a negative control into the test tube. The negative control for T2/HT2 is the ***aokin*** *EasyExtractBuffer* ***T2/HT2*** ② as prepared above.
10.3. Add 15 µL of ***T2/HT2*** *Reagent (**BLACK** cap)* ⑤ into the test tube.
10.4. Mix well/vortex (without spilling).
10.5. Obtain background measurement reading. Wait a minimum of 1 minute and a maximum of 15 minutes between mixing and reading.
10.6. Add 20 µL of ***T2/HT2*** *Tracer (**YELLOW** cap)* ⑤ into the first test tube.
10.7. Mix well.
10.8. Incubate for 60 seconds.
10.9. Obtain tracer measurement

Note: Run negative controls in triplicate.

11. Daily Check II-A:

a. Use liquid positive control in Step 8.2

b. Software: Choose *Daily Check II, Liquid standard* in the user interface or *Recovery rate at given T°C* Sheet in particular excel version of software

11.1. Pipette 765 µl of ***aokin****ReactionBuffer* ④ into a test tube.
11.2. Add 300 µL of a positive control into the test tube. The positive control is a liquid standard diluted in prepared Negative control T2/HT2.
Respectively dilute included **T2-Toxin 8 ng/mL** in Negative control T2/HT2, use 40 to 220 µL within the assay, fill up with Negative control T2/HT2 (4 mL of prepared ***aokin*** *EasyExtractBuffer* ***T2/HT2*** ② diluted with 8 mL of ***aokin*** *EasyPrecipitationBuffer* **T2/HT2** Ⓟ) for overall 300 µL sample volume.
(Example: 100 µL **T2-Toxin 8 ng/mL** + 200 µL Negative control T2/HT2 = 2,67 ng/mL T2-Toxin within the assay)
11.3. Add 15 µL of ***T2/HT2*** *Reagent (**BLACK** cap)* ⑤ into the test tube.
11.4. Mix well/vortex (without spilling).
11.5. Obtain background measurement reading. Wait a minimum of 1 minute and a maximum of 15 minutes between mixing and reading.
11.6. Add 20 µL of ***T2/HT2*** *Tracer (**YELLOW** cap)* ⑤ into the first test tube.
11.7. Mix well.
11.8. Incubate for 60 seconds.
11.9. Obtain tracer measurement

Note: Run positive controls in duplicate.

12. Alternative Daily Check II-B:

a. Use of final extract of a reference matrix material as positive control in Step 8.2

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

12.1. 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.
12.2. Prepare the reference matrix sample according to Steps 4, 5, 6, 7.
12.3. Pipette 765 µl of ***aokin****ReactionBuffer* ④ into a test tube.
12.4. Add 300 µL of the Reference Matrix extract into the test tube.
12.5. Add 15 µL of ***T2/HT2*** *Reagent (**BLACK** cap)* ⑤ into the test tube.
12.6. Mix well/vortex (without spilling).
12.7. Obtain background measurement reading. Wait a minimum of 1 minute and a maximum of 15 minutes between mixing and reading.
12.8. Add 20 µL of ***DON*** *Tracer (**YELLOW** cap)* ⑤ into the first test tube.
12.9. Mix well.
12.10. Incubate for 60 seconds.
12.11. Obtain tracer measurement

Note: Run positive controls in duplicate. Repeat with a different dilution (7.4), if needed.

13. Testing procedure – Sample:

a. Use sample extract in Step 8.2

b. Software: Choose *Analysis* in the user interface or *Analysis at given T°C* Sheet in particular excel version of software

13.1. Pipette 765 µl of ***aokin****ReactionBuffer* ④ into the test tube.
13.2. Add 300 µL of clear sample extract or a control into the test tube.

13.3. Add 15 µL of ***T2/HT2*** *Reagent (****BLACK*** *cap)* ⑤ into the test tube.
13.4. Mix well/vortex (without spilling).
13.5. 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.
13.6. Add 20 µL of ***T2/HT2*** *Tracer (****YELLOW*** cap) ⑤ into the first test tube.
13.7. Mix well.
13.8. Incubate for 60 seconds.
13.9. Obtain tracer measurement of the first sample.
13.10. Repeat steps 12.6 to 12.9 for all test tubes.

Note: Run samples as duplicates. Repeat with a different dilution (8.2), if needed.

14. Test validation

14.1. The mean negative control must read between 170 and 240 mP.
14.2. The positive control must always read lower than the negative control. Expected values of the positive control are 10 mP to 100 mP lower than the negative control.
14.3. 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.
14.4. 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.

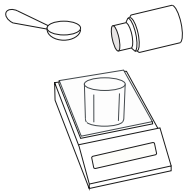
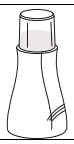
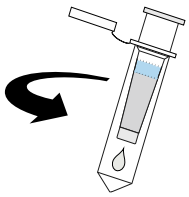
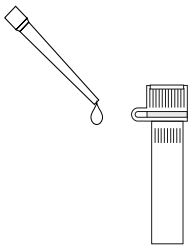
Recommendation: If you notice erroneous measurement values, change the test tube and repeat the measurement after performing measurement of controls. Check that the liquid in the tube is not cloudy and that there are no bubbles after mixing.

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 ensure 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, oats, barley and corn**

Extraction		**Weighing:** 15 g sample 1.5 g ***aokin****ExtractionSalt* ***T2/HT2*** ① 35 mL ***aokin*** *EasyExtractBuffer* ***T2/HT2*** ②
		**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* ***T2/HT2*** ③ column 3 min centrifuge at 5000 x g **2. column:** ≥ 650 µL filtrate of the 1st ***aokin****QuickClean* ③ on 2nd ***aokin****QuickClean* ③ column 3 min centrifuge at 5000 x g or until all liquid passed column use filtrate for measurement
Precipitation		**Precipitation:** 400 µL column filtrate into 800 µL of ***aokin*** *Precipitation buffer* ***T2/HT2*** Ⓟ 5 min centrifuge at > 10.000 x g transfer supernatant into clean 2 mL reaction tube for measurement
Measurement	Mix and read	**Quantification** add into tube: 765 µL ***aokin*** *ReactionBuffer* ④ 300 µL sample 15 µL ***T2/HT2*** *Reagent* ***BLACK*** ⑤ mix well and measure background add ***T2/HT2*** *Tracer* ***YELLOW*** ⑤ 20 µL mix well and measure tracer