INSTRUCTIONS FOR USE

aokin mycontrol OTA IAC

Order No.: MY-IC-M-04

Sample preparation with aokinImmunoClean**M** columns (IAC)

aokin mycontrol OTA *IAC*

Analytical-kit for rapid and quantitative determination of Ochratoxin (OTA).

Materials

aokinmycontrolOTA IAC (Order No.: MY-IC-M-04-100)

Package content

A) *IC Consumables:*
Filter paper
aokinExtractionSalt OTA + spoon
aokinICElute OTA
Reaction tubes 2 mL
IC-Reservoirs (loading reservoirs) 25 mL

B) *IC Liquids:*
aokin*ExtractionSolvent* OTA
aokin*ICDilute 10x*
aokin*ICWash*

C) *Immunoaffinity columns:*
aokin*ImmunoClean* M OTA (Order No.: IC-M-04-100)

D) *Materials for analytical measurement:*
aokinReactionBuffer, Reaction buffer
aokinmycontrolOTA/AFLA SPE, Additive (black cap),
ADD-OTA/AFLA SPE, (for 5 analyses each)
aokinmycontrolOTA, Reagent 1 (yellow cap), F-OTA,
(for 5 analyses each)
aokinmycontrolOTA, Reagent 2 (white cap), A-OTA,
(for 5 analyses each)

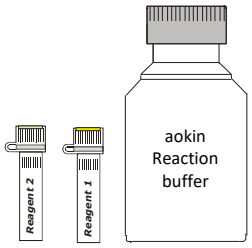


Figure 1: Reagent 1, F-OTA (yellow cap), Reagent 2, A-OTA (white cap) and Reaction buffer (1 L bottle)

E) *Materials for internal quality control:*
aokin*mycontrol* OTA, negative control OTA
(transparent), (for zero value measurements)
aokinmycontrolOTA/AFLA SPE, Additive (black cap),
ADD-OTA/AFLA SPE, (for 5 analyses each)
aokinmycontrolOTA, Reagent 1 (yellow cap), F-OTA,
(for 5 analyses each)
aokinmycontrolOTA, Reagent 2 (white cap), A-OTA,
(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, 2 and aokin*ImmunoClean* M OTA columns 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:
aokinmycontrolOTA (Order No.: MY-IC-M-04-100)

Introduction

aokin*mycontrol***OTA** is a rapid and precise quantitative method for analyzing Ochratoxin (OTA). It has been specifically designed and calibrated for use with **aokin***ImmunoClean* **M** columns. The sample preparation can be completed in 25-45 minutes.
aokinmycontrolOTA is available with a calibration. Please use professional care and check the accuracy by regularly analyzing reference materials (***aokin****ReferenceMatrixMaterials*) and/or standards. Participation in proficiency tests is recommended.
aokin will gladly assist you to customise this test for your specific sample type and application. Please do not hesitate to contact us.

Sample		corn, grain, coffee
Time required for sample preparation		25 to 45 minutes
Time required for measurement		3 minutes
Analysis		
	Measurement range [µg/kg]	
Range 1	1-10	
Range 2	2-20	
Range 3	4-40	

Ochratoxin

Ochratoxin is a mycotoxin produced by fungal molds such as *Aspergillus and Penicillium.* Ochratoxin is known to be commonly present in commodities such as cereals, coffee, dried fruit and red wine. It can accumulate in the body and is considered a human carcinogen. Exposure to ochratoxins through diet can have acute toxicity to kidneys. It is strongly recommended to monitor ochratoxin contamination in food and feed products.

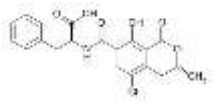


Figure 2: Chemical formula for Ochratoxin A, $C_{20}H_{18}ClNO_6$. Molecular weight: 403.81g/mol

Recommended Accessories

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

	Order No.
aokin*extractor* (food blender)	EX-07-06
aokin*watchbox* (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
Funnel	LB-05-04
Dispensette	LB-08-01
aokin*ReferenceMatrixMaterial*	RMM-04
Reaction tubes, 2mL	LB-05-02
aokin*ICadapter*	LB-08-10-05
aokin*VacuumAdapter*	LB-08-11
Vacuum-pump (diaphragm pump)	LB-04-10
Trap for vacuum-pump (vacuum-bottle)	LB-04-12
Vacuum manifold	LB-04-09
Glass fiber filters, GF/F	LB-04-13-100

Vacuum bottle and funnel for glass fiber filters (Büchner-Funnel)	LB-04-14
pH-indicator strips	LB-08-18
Magnetic stirrer plate (not heated)	LB-04-15
Magnetic stirrer (to degas)	LB-04-16-05
aokin*ICWashTween*	TW-05-1000
PBS	PBS-01-1000

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 quantification range. Use volumes displayed in the *aokin* software.

1. Quality control

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

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, homogenisation, and grinding

The analysis sample is collected, ground, and homo-genised 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* OTA and 20 g extraction solution (22 ml aokinExtractionSolvent OTA at 20°C) respectively 40g (44mL) for absorbent samples like cocoa directly into the extraction beaker.

Figure 3: 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 4: Extracting with the aokinextractor *(blender)*

4. Dilution

Add 166 mL aokinICDilute10x directly into extraction beaker. Shake softly.

5. 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 25 mL). Discard the filter paper and filter cake. Shake/stir the filtrate to ensure homogeneity.

Figure 5: Filtration

Check the pH value. If necessary, readjust the pH value by adding diluted HCl or NaOH to a neutral pH (pH 6.5 - 7.5).

Important: all precipitate has to be removed before loading the column by glass fiber filtration to ensure a good flow through the column (see step 5b).

Figure 6: Filtration through a glass fiber filter using a vacuum (shown as an example for a coffee sample)

5. Clean up with aokinImmunoClean **M**

a) Preparation

Attach the vacuum manifold to a pump. Attach the aokinImmunoClean M column to the vacuum manifold (or alternatively onto an aokin VacuumAdapter) and transfer aokinICWash solution onto the column. Attach the aokin*ICadapter* to the aokinImmunoClean M column and

connect it to the loading reservoir. Pipette aokinICWash into the loading reservoir.

Important: No air bubbles should be visible in the column. Let the aokinICWash solution elute, leaving only a little liquid in the reservoir and so that the aokin*ICadapter* and aokinImmunoClean M column are still filled with liquid.

b) Loading

Load the column with 20 ml of the diluted filtrate. Adjust the vacuum so that a flow rate of approximately **1 ml/min** is produced (total running time approximately 20 minutes). The running speed can be reduced by closing the stopcock, or increased with applying vacuum. Alternatively, a positive pressure from above can be used.

Important: Do not let the aokinImmunoClean M column run dry before, or during loading.

Important: High flow rates lead to a reduced rate of recovery.

c) Washing

Fill 2 x 5 ml aokinICWashTween into the loading reservoir. Adjust the vacuum so that a high flow rate is achieved (running time approximately 2 minutes). The column bed should appear relatively colorless. If this is not observed, the wash step may be repeated. Complete the wash step by adding 2 x 5mL PBS twice to the column. Respectively wash with 2 x 15mL IC-wash, see page 6 till 9. Let the column run dry.

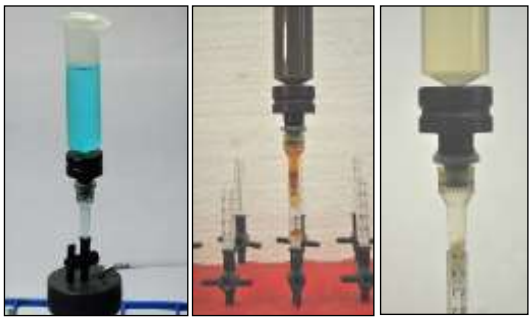


Figure 7: Attachment of reservoir, aokinICadapter and aokinImmunoClean M column to the aokin VacuumAdapter (left), to the vacuum manifold with a coffee sample (middle), and the coffee column after washing with aokin*ICWash or* aokin*ICWashTween and PBS (right).*

d) Elution

Place the column into a collection tube and centrifuge for 1 minute at 1000 x g to remove the residual liquid.

Figure 8: The column is placed into a collection tube before eluent is added, and then the column is centrifuged to remove residual liquid.

Set the column into a new clean collection tube and pipette 300 µl aokinICElute OTA into the column, close (half closed) the column after that with the lid. When closing the column, about 100 µl aokinICElute OTA will be **pushed through the gel**. After incubation for 3 minutes, centrifuge for 1 minute at 3000 x g. The lid must remain on the column during centrifugation. The liquid is present in the collection vessel after centrifugation.

Important note: Eluates of green coffee samples and others may show a precipitate. This leads to increased results. If you see or suspect a precipitation in the eluate than centrifuge eluate at high speed, than pipette supernatant into new collection tube and use for further analysis.

6. Analyzing

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

Important: Gas bubbles can lead to measurement inaccuracies. It is strongly recommended to degas the reaction buffer (aokinReactionBuffer) before each use. This is performed by applying vacuum and stirring for 30 min.

Figure 9: Setup for degassing the reaction buffer (aokinReactionBuffer). This consists of: vacuum pump (hose and vacuum trap, not shown) and the reaction buffer in a bottle with a magnetic stirrer rod on a magnetic stirrer plate.

Please follow detailed instructions for spectrometer use (***aokin****spectrometer****FP470*** & ***aokin****LHW03* Instructions for use).

This includes:

1) Place *Reagents 1* and *2* into position A6 and B6, and place ***Additive*** into position B1 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 OTA*-glass container, filled with *negative control* OTA solution = aokinICElute OTA 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.

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)

aokin**mycontrol OTA **IAC
Standard

Step 1: Extraction

- *Sample mass:* m $_{Sample}$ = 15 g
- *Volume extraction solvent:* V $_{Extraction\ solvent}$ = 188 ml (22 ml $_{EL\ OTA}$ + 16 ml $_{CDilute\ 10x}$ + 150 ml $_{Water}$)
- *Molar mass Ochratoxin:* $MW_{OTA} = 403{,}81\left[\frac{g}{mol}\right]$

Mycotoxin concentration in the sample extract:

$$\boldsymbol{c}\left[\frac{\mu\boldsymbol{mol}}{\boldsymbol{l}}\right]_{\boldsymbol{Extract}} = \frac{m_{Sample}[kg]}{V_{Solvent}[l]*MW_{Mykotoxin}\left[\frac{g}{mol}\right]} * c\left[\frac{\mu g}{kg}\right]_{Sample} = \frac{0.015}{0.188 * 403.81} * c\left[\frac{\mu g}{kg}\right]_{Sample} = 0.00019759 * c\left[\frac{\mu g}{kg}\right]_{Sample}$$

Step 2: Purification

with *aokin* *IC M OTA*

- *Volume sample extract load to the **aokin** IC-M column:* $V_{\ loaded\ sample\ extract}$ = 20 ml
- *Volume "**aokin** Elute" used for the elution of the column:* $V_{\ elute}$ = 0,3 ml

Mycotoxin concentration in the column eluate:

$$\boldsymbol{c}\left[\frac{\boldsymbol{\mu mol}}{\boldsymbol{l}}\right]_{\boldsymbol{Eluate}} = \frac{V_{load\ [ml]}}{V_{elute\ [ml]}} * c\left[\frac{\mu mol}{l}\right]_{Extract} = \frac{20}{0.3} * c\left[\frac{\mu mol}{l}\right]_{Extract} = 66{,}66 * c\left[\frac{\mu mol}{l}\right]_{Extract}$$

Step 3: Measurement

aokin FP 470 / *LHW 03*

- *Sample volume:* $V_{Column\ eluate} = V_{Sample} = 200\ \mu l$
- *Total volume in the cuvette:* $V_{Cuvette} = 2600\ \mu l$

Mycotoxin concentration in the cuvette:

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

Conversion factor: Extraction, Purification and Measurement

It follows the conversion factor from 1 to 3 above:

$$\boldsymbol{c}\left[\frac{\mu\,\boldsymbol{mol}}{\boldsymbol{l}}\right]_{\boldsymbol{Cuvette}} = 0.00019759 * 66{,}66 * 0.0769 * c\left[\frac{\mu g}{kg}\right]_{Sample} = 0.001013 * c\left[\frac{\mu g}{kg}\right]_{Sample} \quad \text{or}$$

$$\boldsymbol{c}\left[\frac{\mu\,\boldsymbol{g}}{\boldsymbol{kg}}\right]_{\boldsymbol{Sample}} = \frac{1}{1.013} * c\left[\frac{nmol}{l}\right]_{Cuvette} = \mathbf{0.987} * \boldsymbol{c}\left[\frac{\boldsymbol{nmol}}{\boldsymbol{l}}\right]_{\boldsymbol{Cuvette}}$$

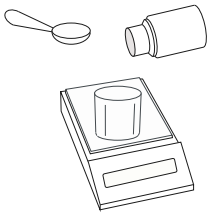
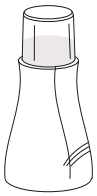
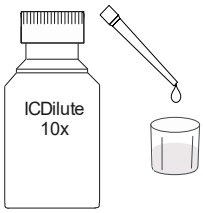
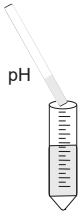
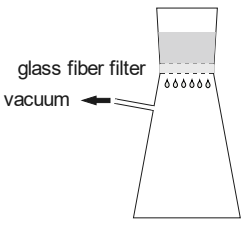
Ochratoxin / standard samples:

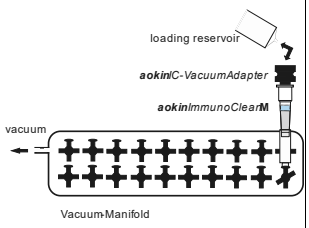
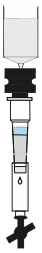
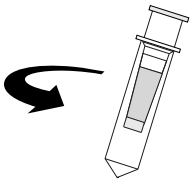
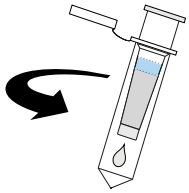
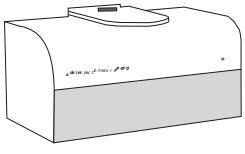
- Recommended for standard types of samples:
  wheat, corn, feeds, nuts, paprika, green and roasted coffee
- Not suitable for absorbent samples → please use the sample preparation for **absorbent samples** (***aokin**mycontrol* ***OTA Absorbent***)
- Not suitable for colored samples → please use the sample preparation for **special matrices** (***aokin**mycontrol* ***OTA Special Matrix***)

***aokin**mycontrol OTA*
Standard

Procedure:

Extraction

	**Weighing:** 15 g sample 1,5 g ***aokin**ExtractionSalt* ***OTA*** 22 mL ***aokin**ExtractionSolvent* ***OTA***
	**Extraction:** 3,5 min mixing with ***aokin**watchbox*
	166 ml ***aokinIC**Dilute 10x*
	**Filtration:** collect filtrate, (discard filter cake)
	check and adjust to 6.5 - 7.5 pH, neutralize if necessary by adding NaOH or HCl
	filtrate through a glass fiber filter (optional)

Purification			set up column
		20 ml	filtrate, 1 drop / second (slow flow rate)
		2 x 15 ml	***aokin****ICWash*
		1 min	centrifuge at 1000 x g
		**Elution:** 300 µl 3 min 1 min	***aokin****ICElute* ***OTA*** incubation with lid on centrifuge at 3000 x g, use eluate for measurement
Measurement		**Automatic Analyse (FP470 / LHW03):** 2000 µl 200 µl 200 µl 100 µl 100 µl	place the 2ml reaction tube in the sample holder of the *LHW03* ***aokin*** *Reaction buffer* ***aokin****mycontrol* ***OTA*** *Additive* sample *(diluted 1:1 - RANGE 1)* *(diluted 1:2 - RANGE 2)* *(diluted 1:4 - RANGE 3)* ***aokin****mycontrol* ***OTA*** Reagent 1 ***aokin****mycontrol* ***OTA*** Reagent 2

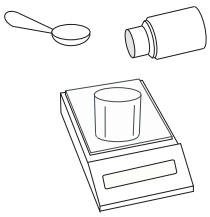
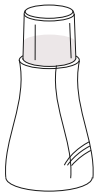
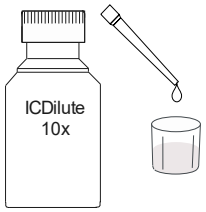
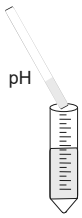
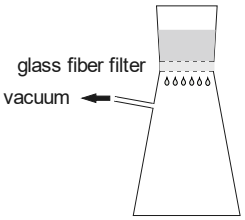
OTA = Ochratoxin, Conversion factor: 1 nmol OTA/l in cuvette = 0,987 µg/kg in sample

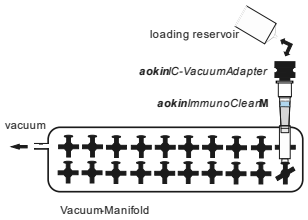
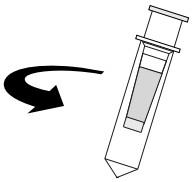
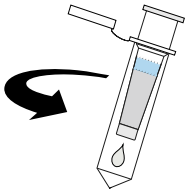
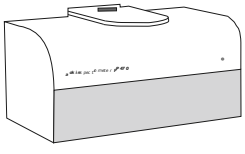
Ochratoxin / absorbent samples:

- Recommended for absorbent samples like **cocoa**
- Not suitable for stadard samples → please use the sample preparation for **standard samples** (***aokin****mycontrol* ***OTA Standard***)
- Not suitable for colored samples → please use the sample preparation for **special matrices** (***aokin****mycontrol* ***OTA Special Matrix***)

aokin*mycontrol OTA*
Absorbent

Procedure:

Extraction		**Weighing:** 15 g sample 1,5 g ***aokin****ExtractionSalt* ***OTA*** 44 mL ***aokin****ExtractionSolvent* ***OTA***
		**Extraction:** 3,5 min mixing with ***aokin****watchbox*
		166 ml ***aokinIC****Dilute 10x*
		**Filtration:** collect filtrate, (discard filter cake)
		check and adjust to 6.5 - 7.5 pH, neutralize if necessary by adding NaOH or HCl
		filtrate through a glass fiber filter (optional)

Purification			set up column
		20 ml	filtrate, 1 drop / second (slow flow rate)
		2 x 15 ml	***aokin****ICWash*
		1 min	centrifuge at 1000 x g
		**Elution:** 300 µl 3 min 1 min	***aokin****ICElute* ***OTA*** incubation with lid on centrifuge at 3000 x g, use eluate for measurement
Measurement		**Automatic Analyse (FP470 / LHW03):** 2000 µl 200 µl 200 µl 100 µl 100 µl	place the 2ml reaction tube in the sample holder of the *LHW03* ***aokin*** *Reaction buffer* ***aokin****mycontrol* ***OTA*** *Additive* sample *(diluted 1:1 - RANGE 1)* *(diluted 1:2 - RANGE 2)* *(diluted 1:4 - RANGE 3)* ***aokin****mycontrol* ***OTA*** Reagent 1 ***aokin****mycontrol* ***OTA*** Reagent 2

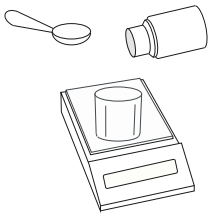
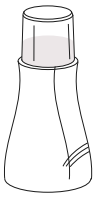
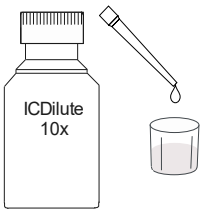
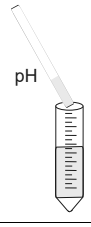
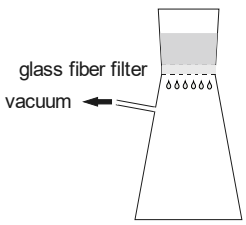
OTA = Ochratoxin, Conversion factor: 1 nmol OTA/l in cuvette = 1,974 µg/kg in sample

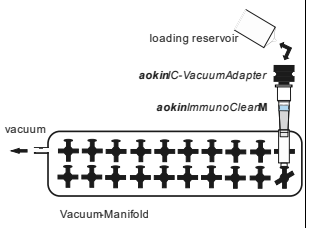
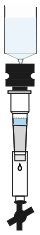
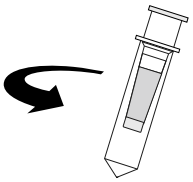
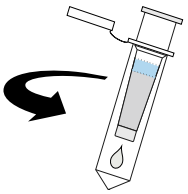
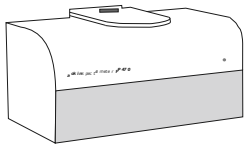
Ochratoxin / special matrix:

- Recommended for colored sample types like **spices (except paprika), some feeds and some nuts**
- Not suitable for stadard samples → please use the sample preparation for **standard samples** (***aokin****mycontrol* ***OTA Standard***)
- Not suitable for absorbent samples → please use the sample preparation for **absorbent samples** (***aokin****mycontrol* ***OTA Absorbent***)

aokin*mycontrol OTA*
Special Matrix

Procedure:

Extraction		**Weighing:** 15 g sample 1,5 g ***aokin****ExtractionSalt* ***OTA*** 22 mL ***aokin****ExtractionSolvent* ***OTA***
		**Extraction:** 3,5 min mixing with ***aokin****watchbox*
		166 ml ***aokinIC****Dilute 10x*
		**Filtration:** collect filtrate, at least 25 ml (discard filter cake)
		check and adjust to 6.5 - 7.5 pH, neutralize if necessary by adding NaOH or HCl
		filtrate through a glass fiber filter (optional)

Purification			set up column
		20 ml	filtrate, 1 drop / second (slow flow rate)
		5 - 20 ml 2 x 5 ml	***aokin**ICWashTween* PBS
		1 min	centrifuge at 1000 x g
		**Elution:** 300 µl 3 min 1 min	***aokin**ICElute **OTA*** incubation with lid on centrifuge at 3000 x g, use eluate for measurement
Measurement		**Automatic Analyse (FP470 / LHW03):** 2000 µl 200 µl 200 µl 100 µl 100 µl	place the 2ml reaction tube in the sample holder of the *LHW03* ***aokin** Reaction buffer* ***aokin**mycontrol **OTA** Additive* sample *(diluted 1:1 - RANGE 1)* *(diluted 1:2 - RANGE 2)* *(diluted 1:4 - RANGE 3)* ***aokin**mycontrol **OTA** Reagent 1* ***aokin**mycontrol **OTA** Reagent 2*

OTA = Ochratoxin, Conversion factor: 1 nmol OTA/l in cuvette = 0,987 µg/kg in sample