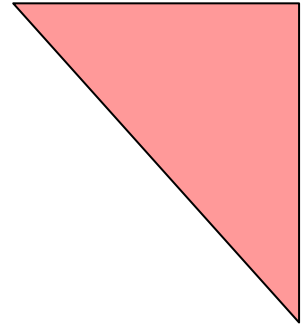


INSTRUCTIONS FOR USE



aokin mycontrol *THC*

Order No.: MY-40

Sample preparation



aokinmycontrol/THC

Analytical-kit for rapid and quantitative determination of Tetrahydrocannabinol (THC).

Materials

aokinmycontrol/THC (Order No.: MY-40-100)

Package content

A) *Materials for sample preparation:*
Filter paper

B) *Materials for analytical measurement:*
aokinReactionBuffer, Reaction buffer for the analysis of Tetrahydrocannabinol (THC)
aokinmycontrol/THC, Reagent 1 (red cap), F-THC, (for 5 analyses each)
aokinmycontrol/THC, Reagent 2 (blue cap), A-THC, (for 5 analyses each)

C) *Materials for internal quality control:*
aokinmycontrol/THC, Blank (transparent), Blank-THC, (for zero value measurements)
aokinmycontrol/THC, Reagent 1 (red cap), F-THC, (for 5 analyses each)
aokinmycontrol/THC, Reagent 2 (blue cap), A-THC, (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.

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:

aokinmycontrol/THC (Order No.: MY-40-100)

Introduction

aokinmycontrol/THC is a rapid and precise quantitative method for analyzing Tetrahydrocannabinol (THC). It has been specifically designed and calibrated for the analysis raw material. Samples in the µg/kg range (ppb = parts per billion range) can be analysed for THC in 10 minutes.

aokinmycontrol/THC is available with a calibration, which has been validated for tea based on cannabis leaves and other raw materials. Please use professional care and check the accuracy by regularly analyzing reference materials (e.g. **aokinReferenceMatrixMaterials**) 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 type: Cannabis leaves and seeds with THC concentrations of 1% (which means 1 g THC/ 100 g sample) or higher | |
| Time required for sample preparation | 5 minutes |
| Time required for measurement | 5 minutes |

Tetrahydrocannabinol

Tetrahydrocannabinol (THC) is the principal psychoactive constituent of cannabis.

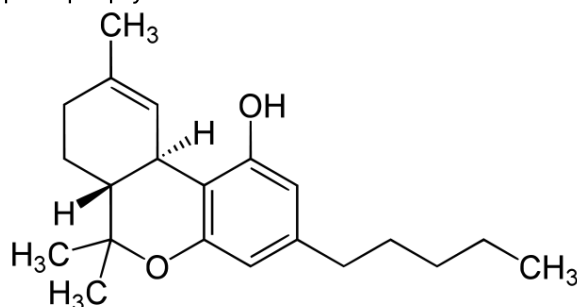


Figure 1: Chemical formula for Tetrahydrocannabinol $C_{21}H_{30}O_2$. Molecular weight: 314,47 g/mol

Recommended Accessories

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

| | |
|---|---------------------|
| aokinextractor (food blender) | Order No.: 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 pipette (1000 µl) | LB-04-05-1000 |
| Pipette tips (1000 µl) | LB-04-08-1000 |
| Funnel | LB-05-04 |
| Dispensette | LB-08-01 |

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, homogenisation, and grinding

The analysis sample is collected, ground, and homogenised according to an approved procedure. Small samples may be ground using the *aokinextractor*.

2. Weighing and extraction

Weigh 15 g of your sample and add 35 mL Methanol (100%) directly into the extraction beaker. Preferentially the exact volume is applied using a dispensette.



Figure 2: Weighing

Close the extraction beaker with the lid (with the blending knives). Start mixing for 3-4 minutes.



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

Alternatively, a magnetic stirrer can be used for a minimum of 10 minutes.

Place the mixed sample in the ultrasonic bath for 15 minutes.

3. 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 2 mL). Discard the filter paper and filter cake. Shake/stir the filtrate to ensure homogeneity.

4. Dilution

Transfer 10 µl of the filtrate into a reaction tube containing 990 µl Methanol (100%).

If needed perform further dilutions with Methanol (100%).

5. Analyzing

Use diluted filtrate for analyzing in the *aokinspectrometerFP470*. 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.
- 5) It is not recommended to do multiple subsequent measurements. Please use a new cuvette every single measurement.

6. 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 **blank**, corresponding to samples free of THC).

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.

CALIBRATION

Calibration is done by measuring standards with the following concentrations of THC in the cuvette: 0 nM, 125 nM, 250 nM, 500 nM, 750 nM.

Preparing those standards:

Transfer 38 μL of the THC-stocksolution (0,1 mg/mL) into a 2 mL Eppendorf tube containing 1202 μL of the solution for dilution. Shake/ stir to ensure homogeneity. (SD 4)

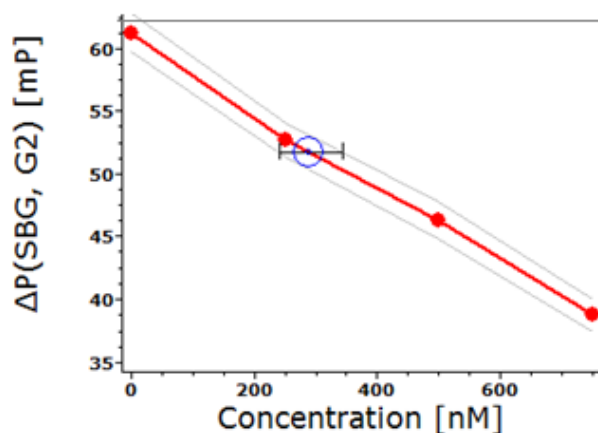
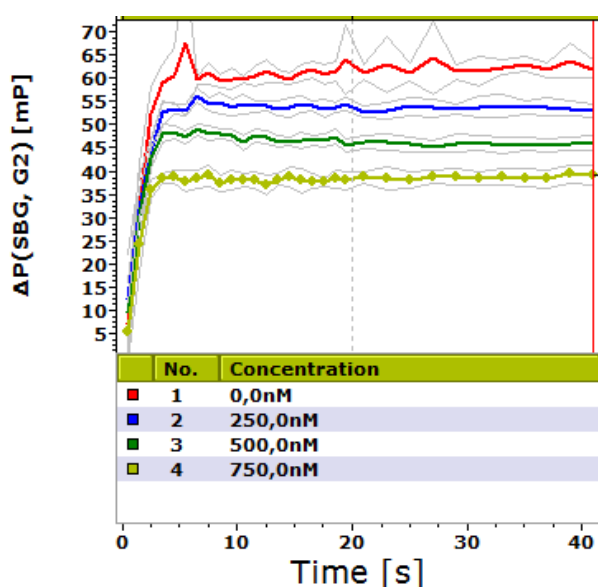
Transfer 600 μL of the solution SD 4 into a 2 mL Eppendorf tube containing 300 μL of the solution for dilution. Shake/ stir to ensure homogeneity. (SD 3)

Transfer 375 μL of the solution SD 3 into a 2 mL Eppendorf tube containing 375 μL of the solution for dilution. Shake/ stir to ensure homogeneity. (SD 2)

Transfer 250 μL of the solution SD 2 into a 2 mL Eppendorf tube containing 250 μL of the solution for dilution. Shake/ stir to ensure homogeneity. (SD 1)

Measure one negative control (0 nM THC) and SD 1 (125 nM THC), SD 2 (250 nM THC), SD 3 (500 nM THC), SD 4 (750 nM THC) to build the calibration.

Illustration:



SUPPLEMENTAL ANALYSIS

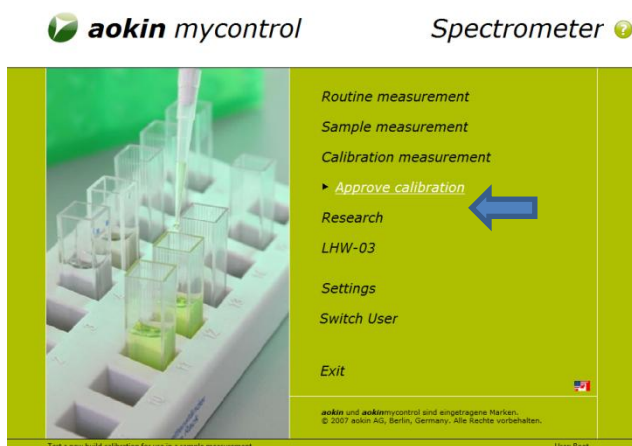
A Negative Control should be run periodically to verify performance of equipment and reagents. A normalization of the calibration is required if measured values are not within a tolerable limit. Use provided negative controls.

NORMALIZATION OF CALIBRATION

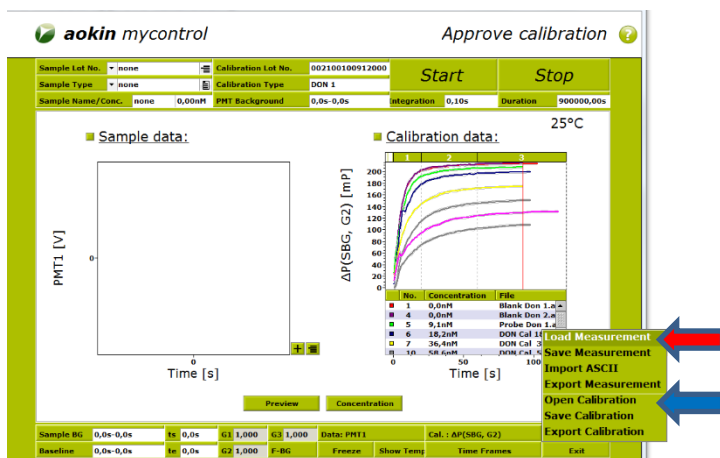
If, after running negative control measurement, a negative control result is obtained, then the system is running normally and no normalization of the calibration is necessary.

If however, the measurement of a negative control shows a positive result, a normalization of the calibration must be performed:

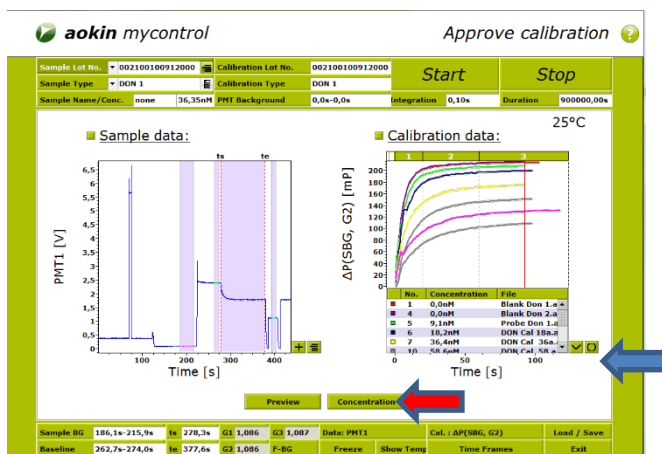
1. Open the window *Approve calibration*



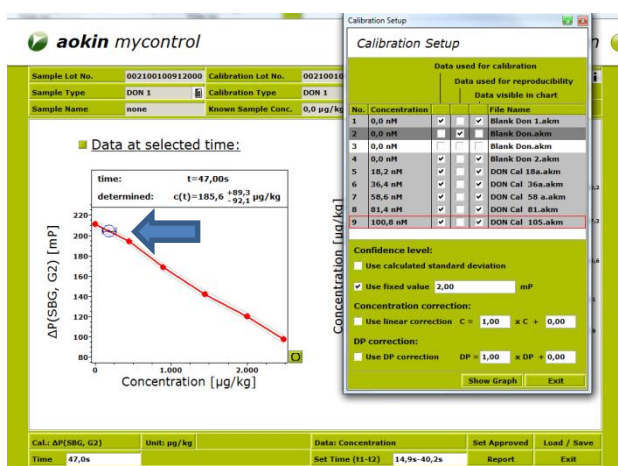
2. Click on the *Load/Save* menu and then click on *Open Calibration* (blue arrow). A file menu will open. Choose a calibration file and click on *Load File*. Then click on the *Load/Save* menu and choose *Load Measurement* (red arrow). A file menu will open. Choose a recent measurement of a negative sample and open it by clicking on *Load File*.



3. Click on the circle shown below by the arrow to open up the *Calibration Setup* window (blue arrow). Then click on *Concentration* (red arrow) to open the *Check Calibration* window.



4. Compare the value of your negative control with the value in the calibration (blue arrow).



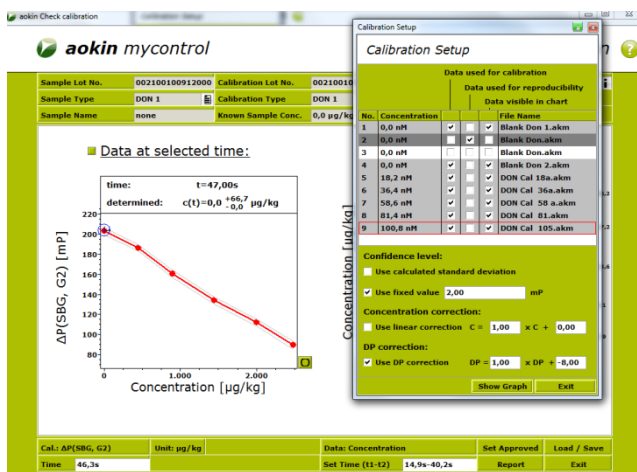
5. In the *Calibration Setup* window, click on *Use DP Correction* in order to offset the calibration. This is recommended ONLY for *small* corrections of the loss of activity of Reagent 2. Next:

- a. Read the mP value of the y-axis of *Data at selected time* graph, ΔP(SBG, G2) of the negative control in the calibration (eg 205 mP).
- b. Read the mP value of your negative control (eg 197 mP).
- c. Calculate the difference and enter as a negative offset in the lower right field of *DP Correction* in the *Calibration Setup* window (see arrow)

Example: if the difference is 8, then enter "-8" into the offset field.

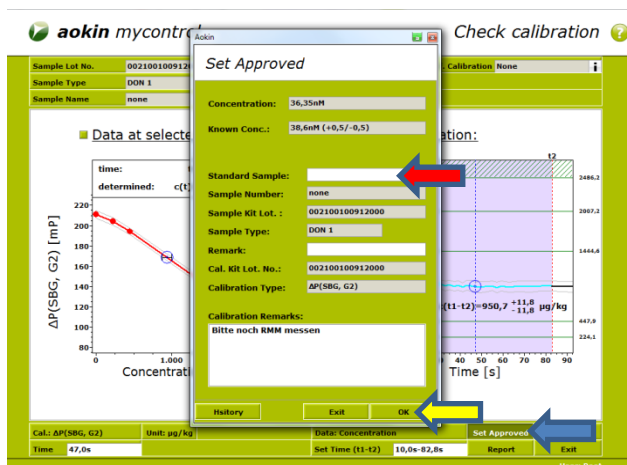
$$DP \text{ correction: } DP = 1,00 \times DP + (-8)$$

- d. Enable the DP Correction by simply clicking anywhere on a blank area of the *Calibration Setup* window.

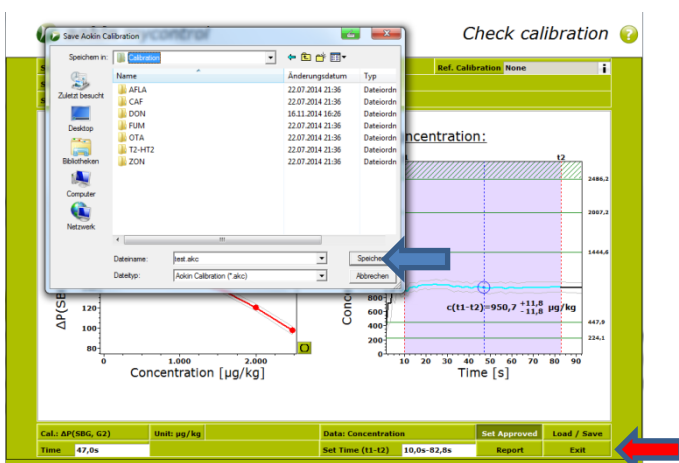


The measured value should now be displayed in the calibration at 0 nM or µg/kg. The other correction factors (i.e. concentration correction) should not be changed without consultation with an aokin technician.

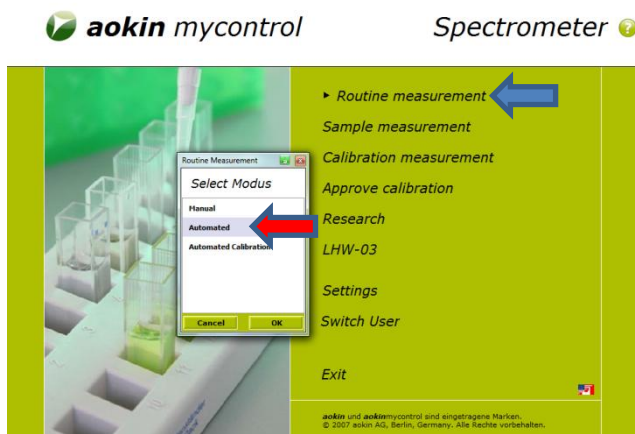
- Click on *Set Approved* (blue arrow), enter the name of the calibration file in the *Standard Sample* field (red arrow) and click *OK* (yellow arrow).



- A *Save Aokin Calibration* window will open. RENAME the calibration with a new revision number and click *Speichern* or *Save* (blue arrow) in order to save the calibration as an approved revised calibration. Afterwards, click on *Exit* (red arrow) to leave this window.

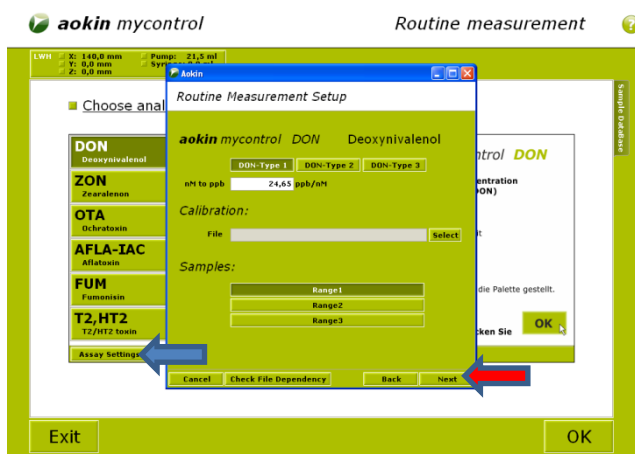


- Next, go to *Routine Measurement* (blue arrow) and Select Modus: *Automated* (red arrow).

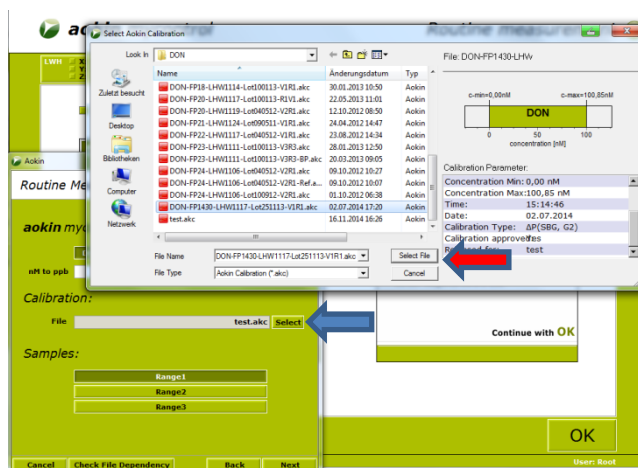


- Choose assay (in this case THC), click on *Assay Settings* (blue arrow) and click on *Next* (red arrow).

- Choose assay (in this case THC), click on *Assay Settings* (blue arrow) and click on *Next* (red arrow).



- Load newly revised and normalized calibration (with new revision number) by clicking *Select* (blue arrow) which opens the *Select Aokin Calibration* window and then clicking on *Select File* (red arrow) to choose the calibration. Click *Next* to close the windows.



The system is now ready to be used. Perform a second negative control to validate the system with the normalized calibration. If the normalization of the calibration does not lead to results within tolerable limits, continue with the software assisted calibration as described in the next section or consult with an **aokin** technician.

SOFTWARE ASSISTED CALIBRATION

This section describes a software assisted full calibration of the system.

A. Preparation of device for calibration:

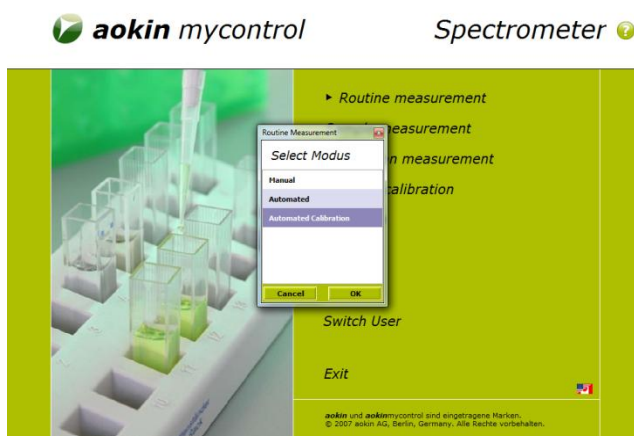
1. Switch on *aokinSpectrometer FP470* and *aokin LHW*.
2. Turn on computer.
3. Open the lid of the *aokinSpectrometer FP470*, unscrew the cover of the polarizer (two black screws) and remove the cover. Close the lid of the spectrometer.
4. Check volume level of the Reaction Buffer and refill if necessary.
5. Fill Clean Bottle and place an empty 2 mL vial in position A1 of the Sample Tray.
6. Place a new cuvette with a clean stirrer into the spectrometer and turn the polarizer to the vertical position V (left).

B. Software settings for Calibration:

1. Start the aokin software.
2. Logon with administrator rights (root).

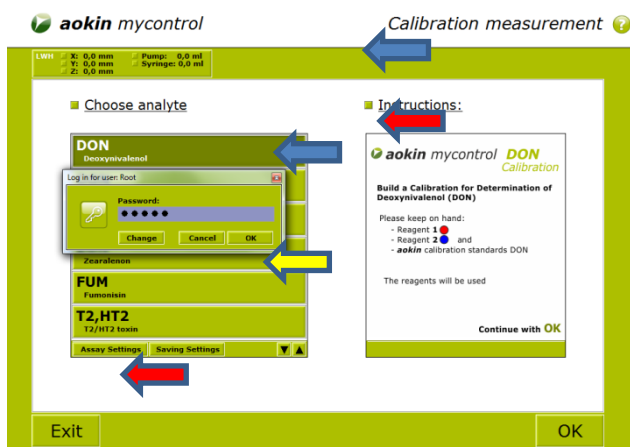


3. Choose Routine Measurement.
4. Click on *Automatic Calibration* and OK (red arrow).



5. Choose THC as your analyte (blue arrow). Click *Assay Settings* (red arrow). The *Log in for user: Root* window will open. Enter the password (default: aokin) and click OK (yellow arrow).

- Choose THC as your analyte (blue arrow). Click Assay Settings (red arrow). The Log in for user: Root window will open. Enter the password (default: aokin) and click OK (yellow arrow).

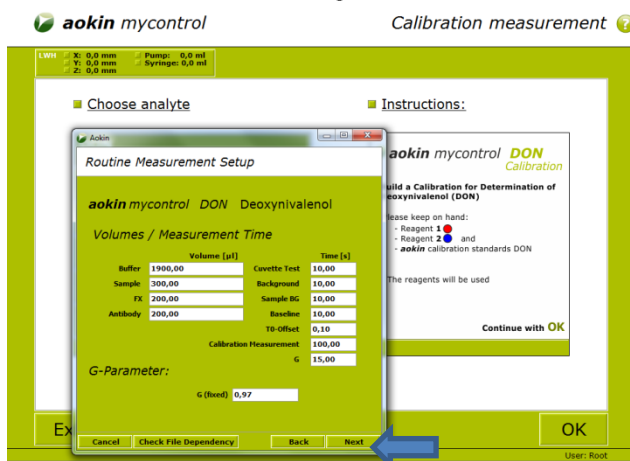


- Choose a Kit Lot No. from the dropdown list or type in a new lot number (blue arrow). Click on Next (red arrow).

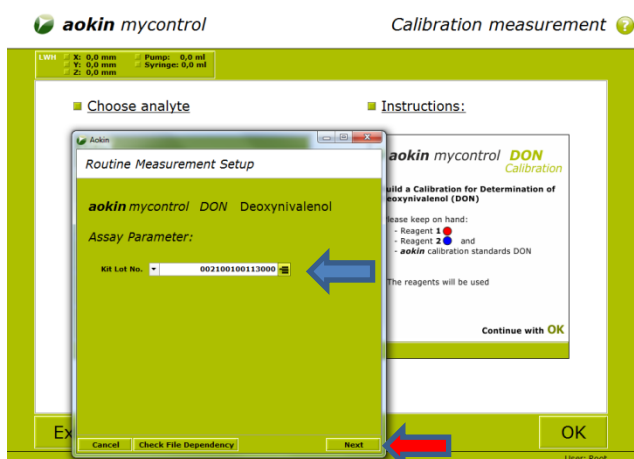


- The Routine Measurement Setup window now displays standard settings for volumes and times. These should not be changed without consulting an aokin technician or when performing research studies.

If the G-Parameter is already known enter it in the G (fixed) field (the value will range between 0.9 and 1.1) otherwise do nothing. Click Next (blue arrow).

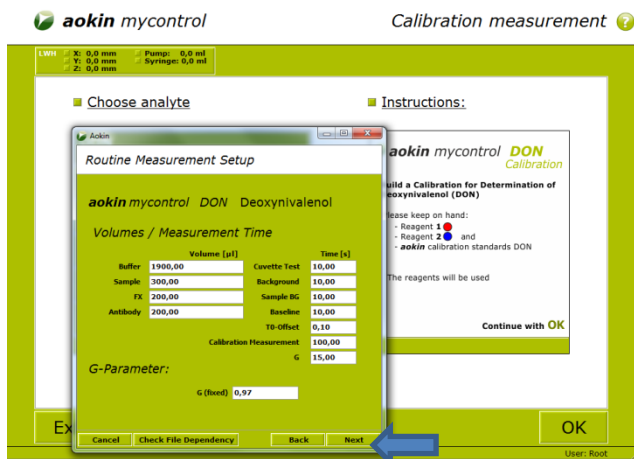


9. Choose a Kit Lot No. from the dropdown list or type in a new lot number (blue arrow). Click on Next (red arrow).

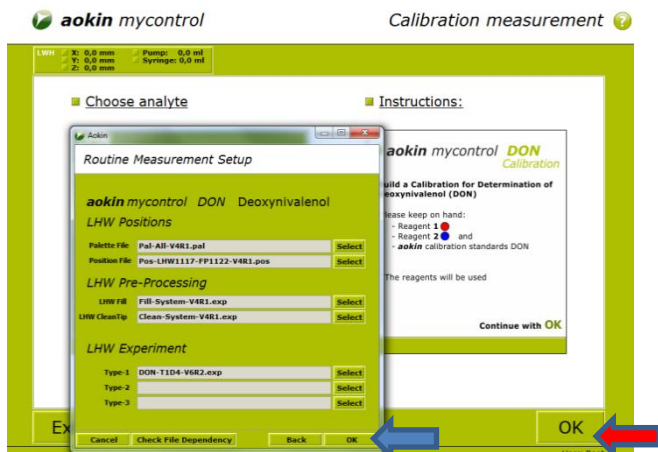


10. The Routine Measurement Setup window now displays standard settings for volumes and times. These should not be changed without consulting an aokin technician or when performing research studies.

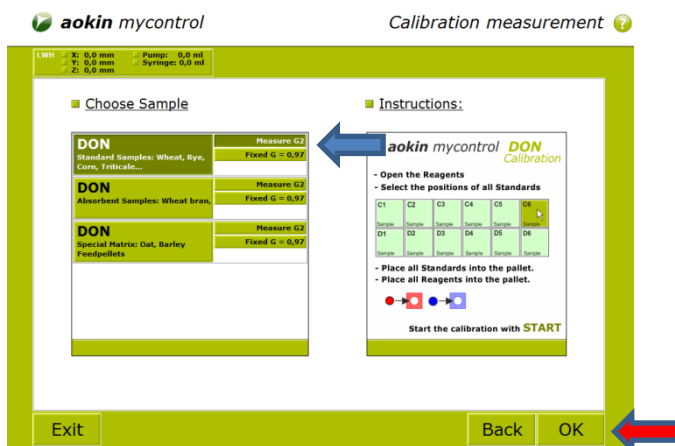
If the G-Parameter is already known enter it in the G (fixed) field (the value will range between 0.9 and 1.1) otherwise do nothing. Click Next (blue arrow).



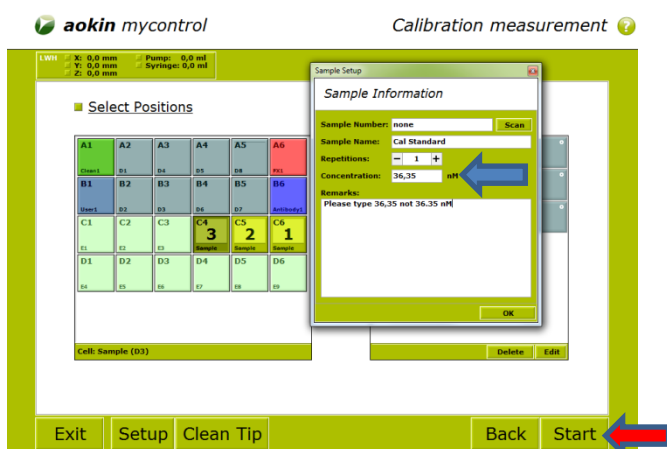
11. The Routine Measurement Setup window now displays experimental files and paths. Do not change anything without consulting an aokin technician. Click OK (blue arrow) to close the Routine Measurement Setup window and again OK (red arrow) to start the calibration.



12. In the *Calibration measurement* window, Choose *Measure G2* (blue arrow) if the G-factor is unknown and *Fixed G* if the G-factor is already known. G-factor determination is described in Step 13. Click *OK* (red arrow) to continue.



13. Vortex a vial of Reagent 1 (Red Cap), remove the cap and place in Position A6. Vortex a vial of Reagent 2 (Blue Cap), remove the cap and place in Position B6. Place 5 standards in any five C-Positions (C-1 through C-6) of the Sample Tray. In *Select Positions*, click on the position of first standard to be measured. The box will become highlighted and a *Sample Setup/Sample Information* window will open. Type in the name of the standard in the *Sample Name* field and enter the concentration in nM (see following table below) in the *Concentration* field (blue arrow). It is important to note that the concentration value entered here is NOT the concentration of the standard in the vial, but rather the concentration of the standard in the cuvette during measurement, which is diluted 13-fold. The *aokinmycontrol* THC protocol uses 200 µL of sample (or standard, in this case) and will have a total reaction volume of 2600 µL.

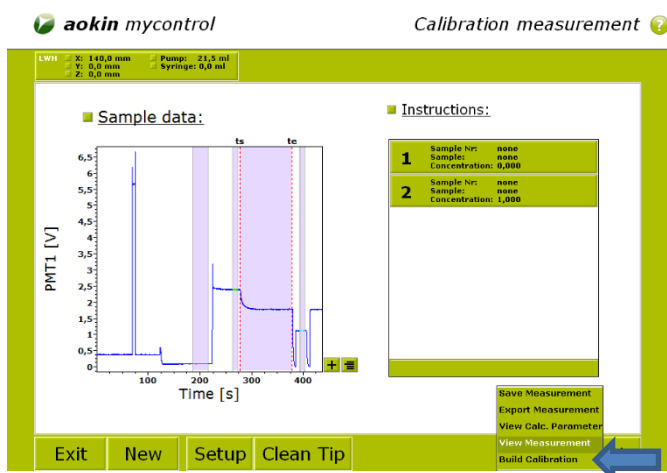


The Table below provides final Cuvette concentrations for aokin standards. If other or a non-aokin standard is used, the final cuvette concentration must be calculated by dividing the nM concentration of the standard by 13 = 200/2600

After all standards have had their respective Names and Final Concentrations entered, click on *Start* (red arrow) to start the calibration measurements (see arrow).

14. In you have previously chosen in Step 9 *Measure G2*, then follow instructions provided on the screen for moving the polarization filter for calibration measurements. The easiest way to move the polarization filter is to open the lid of the spectrometer about ¾ inch (2 cm) just enough to insert your finger to move the polarization filter to the left or right, as instructed by the screen instructions (the LHW arm prevents complete opening of the lid). Alternatively, move the LHW arm using the button on the right side of the LHW just below the emergency stop. Press and HOLD the button for 10 seconds or more until the arm begins to move into the resting position, allowing the lid to be completely opened.

15. After standard measurements have been completed, click on *Build Calibration* (blue arrow). The *Calibration measurement* window opens.

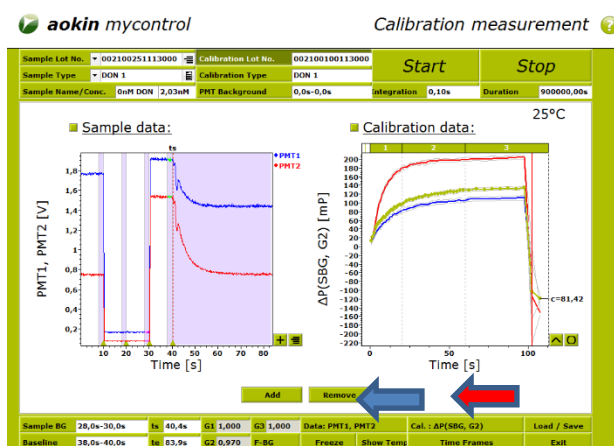


16. Click on the dropdown menu *Data:* and select *PMT1*, *PMT2* for viewing raw data (blue arrow).

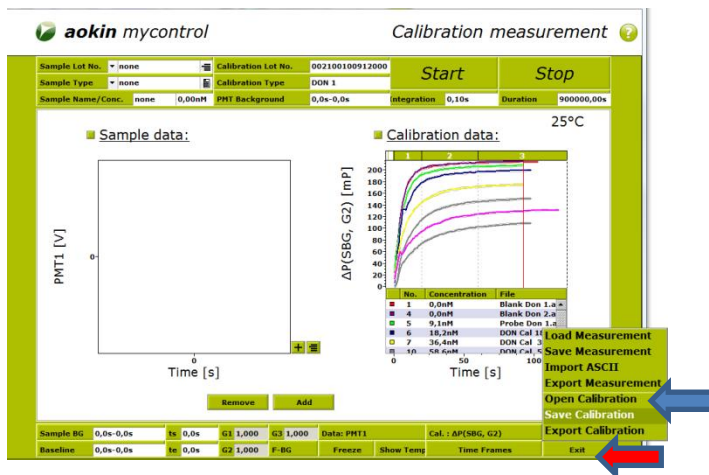
PMT stands for photo multiplier tube. There are two PMTs in the FP470 to simultaneously and independently measure the horizontally polarized emission H and the vertically polarized emission V.

Click on the dropdown menu *Cal:* and select $\Delta P(SBG, G2)$ (red arrow).

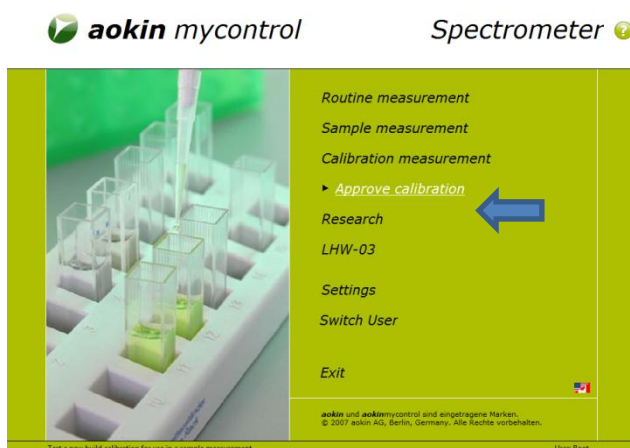
$\Delta P(SBG, G2)$ represents the difference (change) between the P value of the free fluorescent dye (Reagent 1) and the bound P value during the reaction. SBG represents a SampleBackGround correction value. G2 is the G-factor correction, which is necessary for normalizing the outputs of the two PMTs.



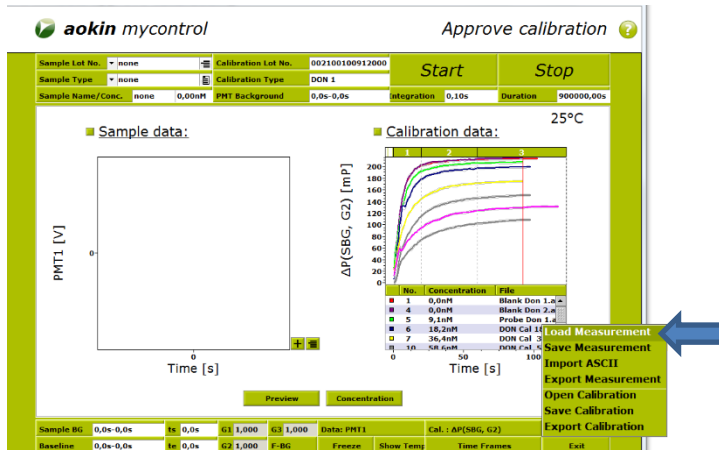
17. To save the calibration, click on *Load/Save* to open the dropdown menu and select *Save Calibration* (blue arrow). To leave the window, click *Exit* (red arrow).



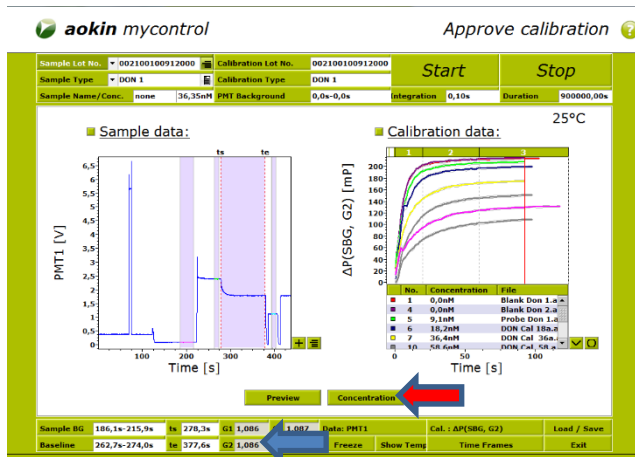
18. Open the window *Approve calibration*



19. Open a measurement file of a middle-value standard (do NOT choose the negative control or the maximum concentration standard) by clicking on *Load/Save* and selecting *Load Measurement* from the dropdown menu. A File Manager window will appear and should open to C:\aokinData\Measurements, where all measurement data files are stored. If the File Manager opens to another location other than C:\aokinData\Measurements, change the file location to C:\aokinData\Measurements to retrieve the measurement file.

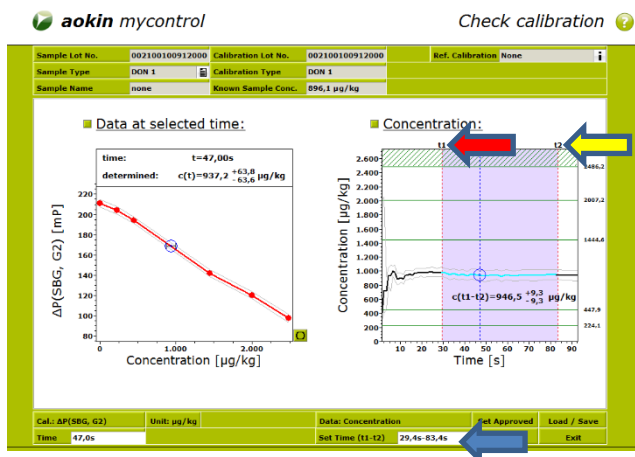


20. Write down as a written note the G2 value of this measurement (blue arrow). Save this value for Step 23.

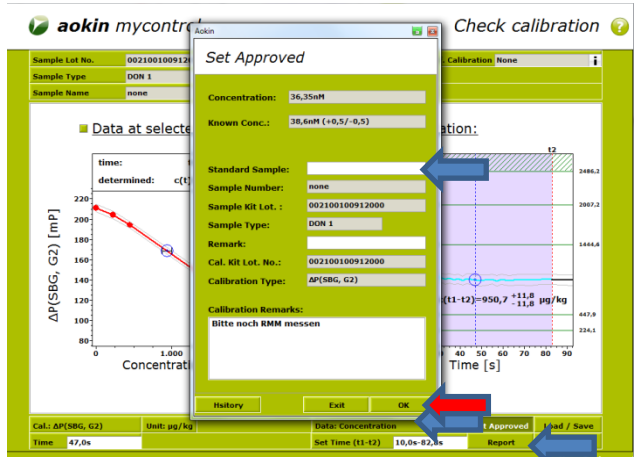


Click on *Concentration* (red arrow). A *Check Calibration* window will open.

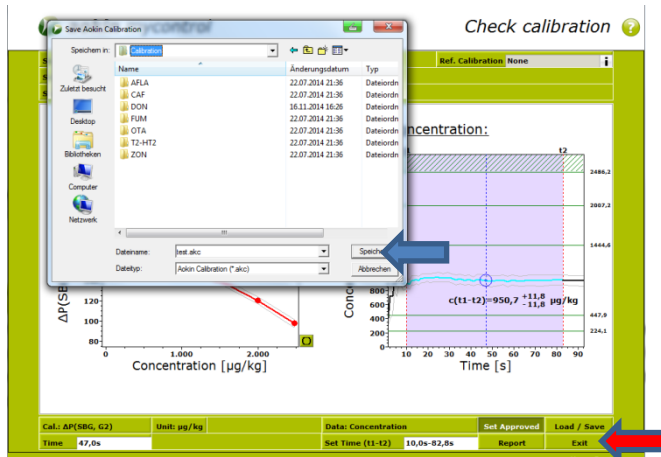
21. To define the time range used for calculating the concentration, click on *Set Time* (blue arrow). Review the *Concentration Graph* on the right side of the screen and note a *Time 1* and a *Time 2* where the concentration is constant over time. Using the left mouse button, click on the *Concentration graph* at your selected *Time 1* (t1) (red arrow) and drag the mouse cursor to your selected *Time 2* (t2) (yellow arrow). Alternatively, *Time 1* and *Time 2* may be entered manually in the field *Set Time* (IMPORTANT: use a comma instead of a period as a decimal point.)



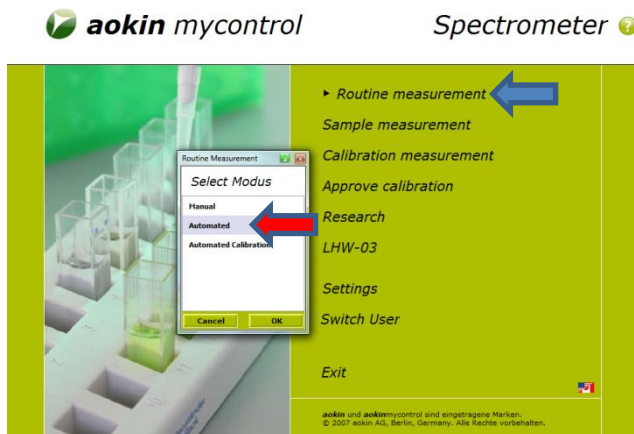
22. Click on *Set Approved*, which opens a new *Set Approved* window. Enter an identifying Name in the *Standard Sample* field (blue arrow) and then click *OK* (red arrow).



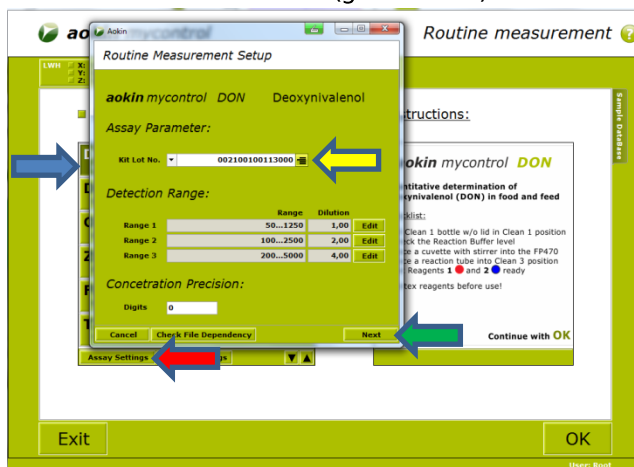
23. A *Save Aokin Calibration* window will open. Name the file and click on *Save* or *Speichen* (blue arrow). The calibration is finished. Click on *Exit* (red arrow) to leave the window.



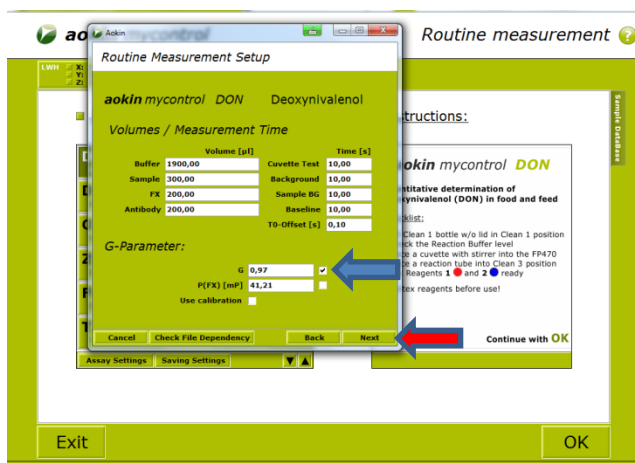
24. Click on *Routine Measurement* (blue arrow) and in the *Select Modus* window select *Automated* (red arrow) and click on *OK*.



25. Select the correct mycotoxin for your assay (blue arrow) and click on *Assay Settings* (red arrow) to open the *Routine Measurement Setup* window. Type in the Kit Lot No. (yellow arrow) used for the calibration and click on *Next* (green arrow).



26. Type in the correct G-factor from the calibration in Step 17 (blue arrow) and click on *Next* (red arrow).



27. Click on the *Select* button to the right of the *Calibration: File* field (blue arrow). The *Select Aokin Calibration* file manager opens. Select the calibration file that has just been saved by clicking and highlighting with the mouse. Check that the calibration is appearing as having been *approved* (yellow arrow). Click on the button *Select File* (red arrow) and then click on *OK*.

28. The system is now ready to be used.

STORAGE CONDITIONS AND PRECAUTIONS:

Storage Conditions: Reagents 1 and 2 must be stored at temperature of +4°C. All other components may be stored at room temperature. Do not freeze any reagents.

Precautions: Do not use the test kits beyond the noted expiration date.

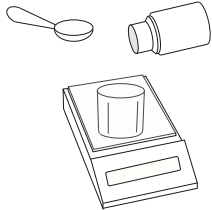
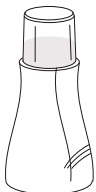


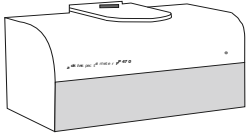
Prolonged exposure to high temperatures may adversely affect the test results. Use of the instrument in extremely humid conditions is not recommended.

Tetrahydrocannabinol / standard samples:

- Recommended for non potent samples

🟢 **aokinmycontrol THC**

Procedure: ***in industrial and food grade hemp samples***

| | | |
|--------------|---|--|
| Extraction |  | Weighing: 15 g sample 35 mL Methanol (100%) |
| |  | Extraction: 3,5 min mixing with aokinwatchbox 15 min ultrasonic bath at room temperature |
| |  | Filtration: collect filtrate, at least 2 ml (discard filter cake) |
| Purification |  | Dilution: 10 µl filtrate 990 µl Methanol (100%) If needed, further dilutions with Methanol. |
| Measurement |  | Automatic Analyse (FP470 / LHW03) place the 2ml-reaction tube in the sample holder of the LHW03 2200 µl aokinReactionBuffer THC 200 µl sample (diluted 1:1 - RANGE 1) (diluted 1:2 - RANGE 2) (diluted 1:4 - RANGE 3) 100 µl aokinmycontrol THC Reagent 1 100 µl aokinmycontrol THC Reagent 2 |

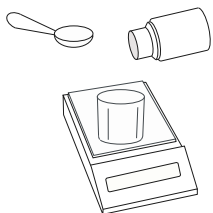
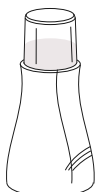


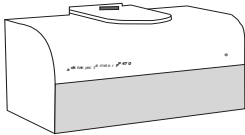
THC = Tetrahydrocannabinol, Conversion factor: 1 nmol THC/l in cuvette = 9,538 µg/kg
the dilution factor of 100 is not included

Tetrahydrocannabinol / potent samples:

- Recommended for potent samples

 **aokinmycontrol** *THC*
in medicinal hemp

Procedure:

| | | |
|--------------|---|--|
| Extraction |  | Weighing: 0.1 g sample 2 mL Methanol (100%) |
| |  | Extraction: 3,5 min mixing with <i>aokinwatchbox</i> 15 min ultrasonic bath at room temperature |
| |  | Separation: Centrifuge at > 10.000 x g |
| Purification |  | Dilution: 10 µl supernatant 990 µl Methanol (100%) If needed, further dilutions with Methanol. |
| Measurement |  | Automatic Analyse (FP470 / LHW03) place the 2ml-reaction tube in the sample holder of the <i>LHW03</i> 2200 µl <i>aokinReactionBuffer THC</i> 200 µl sample (diluted 1:1 - RANGE 1) (diluted 1:2 - RANGE 2) (diluted 1:4 - RANGE 3) 100 µl <i>aokinmycontrol THC</i> Reagent 1 100 µl <i>aokinmycontrol THC</i> Reagent 2 |

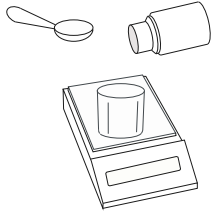

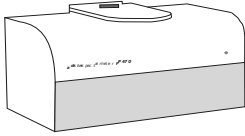
THC = Tetrahydrocannabinol, Conversion factor: 1 nmol THC/l in cuvette = 81,757 µg/kg
the dilution factor (DF) of 100 is not included * DF of further dilutions

Tetrahydrocannabinol / oil samples:

- Recommended for oil samples which are **not** solvable in methanol

aokinmycontrol *THC* in hemp oil (food grade)

Procedure:

| | | |
|--------------|---|--|
| Extraction |  | Weighing: 0.1 mL sample 1.0 mL Methanol (100%) |
| | | Extraction: 5 min mixing with <i>vortex device</i> |
| Purification |  | Dilution: 10 µl Sample in methanol 990 µl Methanol (100%) If needed, further dilutions with Methanol. |
| Measurement |  | Automatic Analyse (FP470 / LHW03) place the 2ml-reaction tube in the sample holder of the <i>LHW03</i> 2200 µl <i>aokinReactionBuffer THC</i> 200 µl sample (diluted 1:1 - RANGE 1) (diluted 1:2 - RANGE 2) (diluted 1:4 - RANGE 3) 100 µl <i>aokinmycontrol THC</i> Reagent 1 100 µl <i>aokinmycontrol THC</i> Reagent 2 |

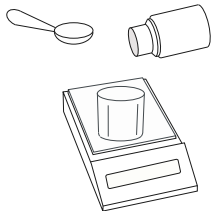

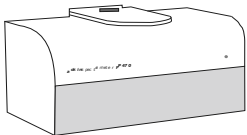
THC = Tetrahydrocannabinol, Conversion factor: 1 nmol THC/l in cuvette = 40,879µg/kg
the dilution factor (DF) of 100 is not included * DF of further dilutions

Tetrahydrocannabinol / dense oil samples:

- Recommended for dense oil samples which are solvable in methanol

aokinmycontrol *THC* in CBD oil

Procedure:

| | | |
|--------------|---|--|
| Extraction |  | Weighing: 0.1 g sample 1.0 mL Methanol (100%) |
| | | Extraction: 5 min mixing with <i>vortex device</i> |
| Purification |  | Dilution: 10 µl Sample in methanol 990 µl Methanol (100%) If needed, further dilutions with Methanol. |
| Measurement |  | Automatic Analyse (FP470 / LHW03) place the 2ml-reaction tube in the sample holder of the <i>LHW03</i> 2200 µl <i>aokinReactionBuffer THC</i> 200 µl sample (diluted 1:1 - RANGE 1) (diluted 1:2 - RANGE 2) (diluted 1:4 - RANGE 3) 100 µl <i>aokinmycontrol THC</i> Reagent 1 100 µl <i>aokinmycontrol THC</i> Reagent 2 |

THC = Tetrahydrocannabinol, Conversion factor: 1 nmol THC/l in cuvette = 10,547µg/kg
the dilution factor (DF) of 100 is not included * DF of further dilutions