INSTRUCTIONS FOR USE

aokin mycontrol THC

Order No.: MYS-40

Quantitative determination of **Tetrahydrocannabinol**

Designed for use with ***aokin*** **FP analyzer**.

Package content:

Order No. MYS-40
aokin*mycontrol* ***THC***

Order No	**Material**	**Content**	**Application**	**Storage**
MYS-40-20 for 20 measurements	***aokin****mycontrol* ***THC*** Reagents for determination of Tetrahydrocannabinol (THC)	***aokin*** *ReactionBuffer* ***THC*** ④, 1 bottle ***THC*** *Reagent* ⑤, 1 unit ***THC*** *Tracer* ⑤, 1 unit ***THC*** *positive control, Standard* ***THC*** ⑤, 1 unit	Measurement on ***aokin*** **FP analyzer**	Refrigerated Do not freeze
MYS-40-100 for 100 measurements	***aokin****mycontrol* ***THC*** Reagents for determination of Tetrahydrocannabinol (THC)	***aokin*** *ReactionBuffer* ***THC*** ④, 1 bottle ***THC*** *Reagent* ⑤, 5 units ***THC*** *Tracer* ⑤, 5 units ***THC*** *positive control, Standard* ***THC*** ⑤, 2 units	Measurement on ***aokin*** **FP analyzer**	Refrigerated Do not freeze

Analytical-kit for quantitative determination of Tetrahydrocannabinol (THC) in hemp, hemp oil or CBD oil.

Materials

All materials provided are precisely weighed and calibrated. Control of the volume and concentration of the individual solutions 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 having different Lot-numbers will lead to erroneous results and is not permitted.

Storage and stability:

aokin *ReactionBuffer* ***THC*** ④ has to be brought to room temperature (best 18 - 26°C) for usage, the ***aokin*** *ReactionBuffer* ***THC*** ④ is stable for 6 months without refrigeration (15° - 30°C).

Kit reagents (***THC*** *Reagent*, ***THC*** *Tracer*, *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 – 200 µL, 100-1000 µL and related pipette tips, 2 mL collection tubes, tube vortex mixer, centrifuge, timer, ultrasonic bath, thermometer, 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* ***THC*** is a rapid method for quantifying Tetrahydrocannabinol (THC). It has been specifically designed and calibrated for the analysis of hemp derived products, such as hemp food products, CBD oils and formulations and medicinal hemp products. LOD is at 0.001%. Samples can be analysed for THC within 5 minutes.

aokin*mycontrol* ***THC*** is available with a calibration, which has been validated for CBD oil and hemp tea and other hemp products. Validation information is available upon request at info@aokin.com.

FPA 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 with the single tube assay or on every microtiter plate, respectively. Avoid temperature variations of more than 1°C during one determination. Mix well with a vortexer and wait 60 s before taking the value. Be careful to avoid bubbles when pipetting into microtiter plates.

Negative control THC = ***Methanol***

Positive controls:

Positive controls are recommended as an additional measure 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 THC ⑤ = Standards diluted in ***Methanol***
THC-D3 100 ng/mL in Negative control THC, use 20 to 50 µL within the assay, fill up with Negative control THC (Methanol) for overall 100 µL sample.
(Example: 40 µL **THC-D3 100 ng/mL** + 60 µL Negative control THC (Methanol) = 40 ng/mL THC within the assay)

Tetrahydrocannabinol

Tetrahydrocannabinol (THC) is the principal psychoactive constituent of cannabis. International and national drug control laws apply. As a consequence, it is strongly recommended to monitor THC content in all medicinal, recreational and herbal cannabis and all hemp based products.

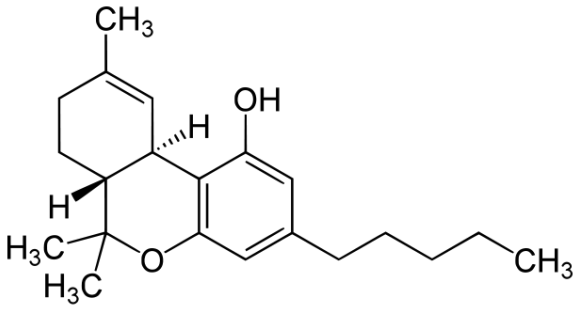


Figure 1: Chemical formula of Tetrahydrocannabinol ($C_{21}H_{30}O_2$. Molecular weight: 314.47 g/mol)

Standard sample preparation for hemp tea, medicinal or recreational hemp, hemp oil or CBD oil (Page 7-10)

1. **Preparation**
   Allow ***aokin*** *ReactionBuffer* ***THC*** ④ to reach room temperature (15°C to 30°C) before use.

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.

Weighing and extraction

4.1. **Hemp tea** (Steps ① + ② + ③)
4.1.1. Weigh 15 g sample and add 35 mL Methanol
4.1.2. Blend for 3-5 minutes in a small food blender
4.1.3. Place sample in an ultrasonic bath at room temperature for 15 minutes
4.1.4. Filter through a fast flow fluted filter. Discard filter cake, use filtrate for the next step. Alternatively centrifuge at >10000 x g for 5 minutes, use supernatant for the next step.
4.1.5. Dilute by pipetting 10 µL of the filtrate into 990 µL Methanol.

4.2. **Medicinal or recreational hemp** (Steps ① + ② + ③)
4.2.1. Weigh 0.1 g sample and add 2 mL Methanol
4.2.2. Mix well with vortex-mixer for 5 minutes
4.2.3. Centrifuge at >10000 x g for 5 minutes
4.2.4. Dilute by pipetting 10 µL of the filtrate into 990 µL Methanol.

4.3. **Hemp oil (food grade)** (Steps ① + ② + ③)
4.3.1. Weigh 0.1 g sample and add 1 mL Methanol
4.3.2. Mix well with vortex-mixer for 5 minutes
4.3.3. Centrifuge at >10.000 x g for 5 minutes
4.3.4. Phase separation occurs. Use Methanol phase for the next step
4.3.5. Dilute by pipetting 10 µL of the Methanol phase into 990 µL Methanol.

4.4. **CBD oil** (Steps ① + ② + ③)
4.4.1. Weigh 0.1 g sample and add 0.2 mL Methanol
4.4.2. Mix well with vortex-mixer for 5 minutes
4.4.3. Dilute by pipetting 10 µL of the Methanol phase into 990 µL Methanol.

5. **Introduction of general testing procedure** (Steps ④ + ⑤)

5.1. Pipette 970 µl of ***aokin****ReactionBuffer* ***THC*** ④ into the test tube.
5.2. Add 100 µL of clear sample extract (if necessery fill up with Negative control THC if sample extract is too concentrated, example: 50 µL sample + 50 µL Negative control THC for overall 100 µL sample volume) or a control into the test tube.

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

5.3. Add 10 µL of ***THC*** *Reagent* (***BLUE*** *cap*) ⑤ into the test tube.
5.4. Mix well/vortex (without spilling).
5.5. Incubate for 5 minutes.
5.6. 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.
5.7. Add 20 µL of ***THC*** *Tracer* (***RED*** cap) ⑤ into the first test tube.
5.8. Mix well.
5.9. Incubate for 60 seconds.
5.10. Obtain tracer measurement of the first sample.
5.11. Repeat steps 5.6 to 5.9 for all test tubes.

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

6. **Data acquisition and analysis**

6.1. **Mix and Read Process**
6.1.1. Mix buffer, sample and reagent in exact volumes and at exactly specified time intervals. Read background polarized fluorescence (automatic reading in horizontal and vertical direction).
6.1.2. Add tracer, incubate for exactly the specified time. Read emission values (automatic reading in horizontal and vertical direction).

6.2. **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 = (ΔH-ΔV)/ (ΔH+ΔV).

6.3. **Software: direct use of excel worksheet - *aokin mycontrol* version of software – alternatively use user interface**

Advice when directly using the excel worksheet: Use a copy of an 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 analyser
- 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

7. Daily Check I:
a. Use negative control (= Methanol) in Step 5.2
b. Software: Choose *Daily Check I* in the user interface or alternatively *Offset at given T°C* Sheet in particular excel version of software

7.1. Pipette 970 µl of ***aokin*** *ReactionBuffer* ***THC*** ④ into a test tube.
7.2. Add 100 µL of a negative control into the test tube. The negative control for THC is Methanol.
7.3. Add 10 µL of ***THC*** *Reagent* (***BLUE*** *cap*) ⑤ into the test tube.
7.4. Mix well/vortex (without spilling).
7.5. Obtain background measurement reading.
7.6. Add 20 µL of ***THC*** *Tracer* (***RED*** cap) ⑤ into the first test tube.
7.7. Mix well.
7.8. Incubate for 60 seconds.
7.9. Obtain tracer measurement.

Note: Run negative controls in triplicate.

8. Daily Check II-A:
a. Use liquid positive control in Step 5.2
b. Software: Choose *Daily Check II, Liquid standard* in the user interface or alternatively *Recovery rate at given T°C* Sheet in particular excel version of software

8.1. Pipette 970 µl of ***aokin****ReactionBuffer* ***THC*** ④ into a test tube.
8.2. Add 100 µL of a positive control into the test tube. The positive control is a liquid standard diluted in Methanol.
Respectively **THC-D3 100 ng/mL** in Negative control THC, use 20 to 50 µL within the assay, fill up with Negative control THC (Methanol) for overall 100 µL sample.
(Example: 40 µL **THC-D3 100 ng/mL** + 60 µL Negative control THC (Methanol) = 40 ng/mL THC within the assay)
8.3. Add 10 µL of ***THC*** *Reagent* (***BLUE*** *cap*) ⑤ into the test tube.
8.4. Mix well/vortex (without spilling).
8.5. Obtain background measurement reading.
8.6. Add 20 µL of ***THC*** *tracer* (***RED*** cap) ⑤ into the first test tube.
8.7. Mix well.
8.8. Incubate for 60 seconds.
8.9. Obtain tracer measurement.

Note: Run positive controls in duplicate.

9. Alternative Daily Check II-B:
a. Use of a final extract of a reference matrix material as positive control in Step 5.2
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

9.1. Choose a reference matrix sample most similar to your samples.
Example: In case you analyse hemp tea, use a hemp tea reference matrix sample containing a known amount of mycotoxin.
9.2. Prepare the reference matrix sample according to Steps 4, 5, 6.
9.3. Pipette 970 µl of ***aokin*** *ReactionBuffer* ***THC*** ④ into a test tube.
9.4. Add 100 µL of the reference matrix extract into the test tube.
9.5. Add 10 µL of ***THC*** *Reagent* (***BLUE*** *cap*) ⑤ into the test tube.
9.6. Mix well/vortex (without spilling).
9.7. Obtain background measurement reading.
9.8. Add 20 µL of ***THC*** *tracer* (***RED*** cap) ⑤ into the first test tube.
9.9. Mix well.
9.10. Incubate for 60 seconds.
9.11. Obtain tracer measurement.

Note: Run positive controls in duplicate. Repeat with a different dilution (Step 5.2), if needed.

10. Testing procedure – Sample:
a. Use sample extract in in Step 5.2
b. Software: Choose *Analysis* in the user interface or *Analysis at given T°C* Sheet in particular excel version of software

10.1. Pipette 970 µl of ***aokin*** *ReactionBuffer* ***THC*** ④ into the test tube.
10.2. Add 100 µL of clear sample extract or a control into the test tube.
10.3. Add 10 µL of ***THC*** *Reagent* (***BLUE*** *cap*) ⑤ into the test tube.
10.4. Mix well/vortex (without spilling).
10.5. 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.
10.6. Add 20 µL of ***THC*** *tracer* (***RED*** cap) ⑤ into the first test tube.
10.7. Mix well.
10.8. Incubate for 60 seconds.
10.9. Obtain tracer measurement of the first sample.
10.10. Repeat Steps 10.6 to 10.9 for all test tubes.

Note: Run samples as duplicates. Repeat with a different dilution (Step 5.2), if needed.

11. Test validation

11.1. The mean negative control must read between 150 and 220 mP.
11.2. The positive control must always read lower than the negative control. Expected values of the positive control are 10 mP to 60 mP lower than the negative control.
11.3. 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.
11.4. 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.

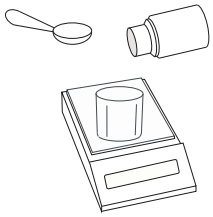
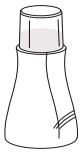
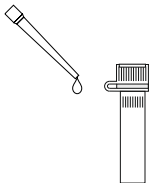
Recommendation: If you notice erroneous measurement values, change the test tube and repeat the measurement after performing measurement of controls. Check that the liquid in the tube is not cloudy and that there are no bubbles after mixing.

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 normalize the readings, this ensures the analytical performance.

Be aware: The general composition of controls and samples should always be identical in salt concentration, pH and solvent ratio.

Hemp tea

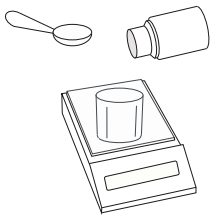
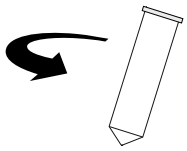
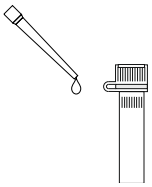
Sample preparation for **industrial hemp, hemp tea, hemp protein**
Δ9-Tetrahydrocannabinol LOD 0,00023 %

Extraction		**Weighing:** **Steps** ① + ② 15 g sample 35 mL Methanol
		**Extraction:** 3-5 min mixing 15 min ultrasonic bath at room temperature
		**Filtration:** collect filtrate (discard filter cake)
Dilution		**Dilution:** **Step** ③ 10 µL filtrate 990 µL Methanol
Measurement	Mix and read	**Quantification** **Steps** ④ + ⑤ add into tube: 970 µL ***aokin****ReactionBuffer* ***THC*** ④ 100 µL sample 10 µL ***THC*** *Reagent* ***BLUE*** ⑤ mix well measure background 20 µL add ***THC*** *Tracer* ***RED*** ⑤ mix well, incubate 60 sec measure tracer

Medicinal hemp

Sample preparation for **medicinal or recreational hemp**

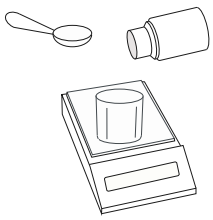
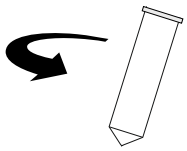
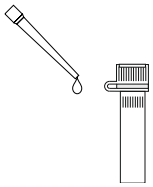
Δ9-Tetrahydrocannabinol LOD 0,002 %

Extraction		**Weighing:** **Steps** ① + ② 0.1 g sample 2 mL Methanol
		**Extraction:** 5 min mixing/vortexing 5 min centrifuge at >10000 x g
Dilution		**Dilution:** **Step** ③ 10 µL filtrate 990 µL Methanol
Measurement	Mix and read	**Quantification** **Steps** ④ + ⑤ add into tube: 970 µL ***aokin****ReactionBuffer* ***THC*** ④ 100 µL sample 10 µL ***THC*** *Reagent* ***BLUE*** ⑤ mix well measure background 20 µL add ***THC*** *Tracer* ***RED*** ⑤ mix well, incubate 60 sec measure tracer

Hemp oil

Sample preparation for **hemp oil (food grade)**

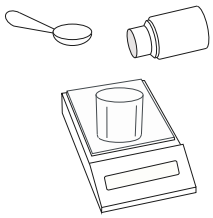
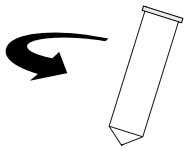
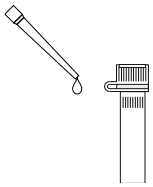
Δ9-Tetrahydrocannabinol LOD 0,001 %

Extraction		**Weighing:** **Steps** ① + ② 0.1 g sample 1 mL Methanol
		**Extraction:** 5 min mixing/vortexing 5 min centrifuge at >10000 x g phase separation occurs use Methanol phase for the next step
Dilution		**Dilution:** **Step** ③ 10 µL Methanol phase 990 µL Methanol
Measurement	Mix and read	**Quantification** **Steps** ④ + ⑤ add into tube: 970 µL ***aokin****ReactionBuffer* ***THC*** ④ 100 µL sample 10 µL ***THC*** *Reagent* ***BLUE*** ⑤ mix well measure background 20 µL add ***THC*** *Tracer* ***RED*** ⑤ mix well, incubate 60 sec measure tracer

CBD oil

Sample preparation for **CBD oil**

Δ9-Tetrahydrocannabinol LOD 0,0003 %

Extraction		**Weighing:** **Steps** ① + ② 0.1 g sample 0.2 mL Methanol
		**Extraction:** 5 min mixing/vortexing
Dilution		**Dilution:** **Step** ③ 10 µL raw sample 990 µL Methanol
Measurement	Mix and read	**Quantification** **Steps** ④ + ⑤ add into tube: 970 µL ***aokin****ReactionBuffer* ***THC*** ④ 100 µL sample 10 µL ***THC*** *Reagent* ***BLUE*** ⑤ mix well measure background 20 µL add ***THC*** *Tracer* ***RED*** ⑤ mix well, incubate 60 sec measure tracer