kaneka

2018.12
Code No. KN-T10C805
第 1 版

Instruction Manual

Kaneka Nucleic Acid Chromatography [Cannabis grass DNA Detection Kit]

Caution

- Keep the reagents in this kit in strict compliance with the storage conditions described in this instruction manual. Be careful of contamination when storing.
- Please wear protective equipment (protective gloves, protective glasses, mask, etc.) before using this kit.
- The specifications of this kit are subject to change without notice.
- For equipment, instruments, reagents, etc. used to prepare PCR solutions and others, follow the instructions specified by the respective manufacturers and distributors.
- It is the customer's responsibility to determine and use the results obtained with this kit. The Company shall not be liable for any damage or loss resulting from the detection or use of the results, whether direct or indirect.
- The customer is responsible for verifying the operating procedures not described in this instruction manual and the validity of the detection results for each sample.

1. Kit Overview

This kit uses a multiplex PCR and a nucleic acid chromatographic test strip to detect cannabis-derived DNA quickly and easily.

2. Principles/Main Features

This kit is based on the principle of nucleic acid chromatography. Nucleic acid amplification is performed by targeting a region common to green plants on the genome and two regions specific to cannabis grass on the chloroplast genome. The amplified product is spread on a test strip, and the color pattern of the line is used to determine whether the sample contains cannabis DNA.

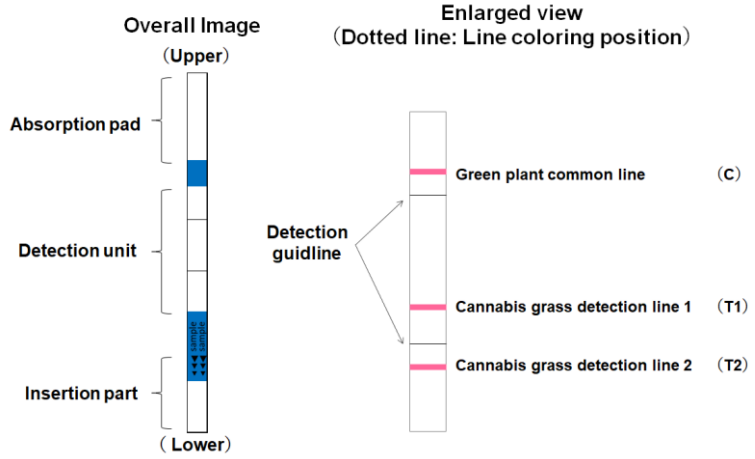
【Main Features of this Kit】

- Testing for Cannabis positivity is possible from samples (cannabis resin, roots, etc.) that do not have the characteristic (bristles, etc.) of cannabis grass.
- No device (gel imaging device, real-time PCR device, etc.) is required for determining results, and it can be determined visually.
- It can perform DNA extraction to result determination within 3 hours.

3． Kit composition/Storage conditions

Component	Contents (20 tests)	Contents (100 tests)	Storage Temp.
Test strip	20 strips	100 strips	Room Temp.
Chase buffer	3.2 mL	16 mL	
DNA extraction Solution A	1 mL	5 mL	
DNA extraction Solution B	20 mL	100 mL	
PCR mix	200 µL	1,000 µL	Frozen (Below -20℃)
Primer mix	100 µL	500 µL	
Positive control DNA	40 µL	200 µL	

Name of test strip parts



【Precautions】

- The test strip may lose its detection performance if left for a long time in a humid environment. After opening the test strip, keep the container lid tightly closed and store it carefully.
- Do not use components from different lots together or use reagents other than those supplied with this kit, as they may not produce proper results.

4． Expiration Date

Stated on the label of each content.

5． How to Use

5.1 Equipment and device required in addition to this Kit

- Micropipette
- Tip for micropipette
- Thermal cycler (Recommended device: LifeECO Thermal Cycler (Bioer Technology))
- 1.5 mL tube
- 0.2 mL tube（For PCR）

・ Small incubator (for 1.5 mL, capable of heating above 95℃)
・ Low speed centrifuge

5.2 Samples

In addition to various parts of cannabis plant, processed products containing cannabis plant can be used as samples of this kit.

Suitable	・Cannabis grass leaves, spikes, stems, seeds, roots (dry or raw, juvenile phase is acceptable) ・Cannabis resin ・Cannabis oil (including plant pieces) ・Cannabis and other plant mixed samples ・Extraction residue after inspection of contained components (use after drying after evaporation of solvent)
Suitable in some conditions	・Combustion residue ・Food (cannabis cookies, etc.)
Not Suitable	・Combustion residue (ashed) ・Cannabis oil (without plant fragments) ・Other samples in bad condition (septic, etc.)

5.3 Sample weight

Weigh a sample volume (generally 1-2 mg) suitable for testing with this kit into a 1.5 mL tube

・Non-seed area: For dried samples, use 1-2 mg.
(In the undried state, the amount of DNA recovered per unit weight tends to decrease.)
・Seeds: We recommend crushing one grain and using the entire amount.
・Cannabis Resin: As it contains PCR inhibitors, we recommend a starting amount of 1 mg or less.
・Cannabis oil: It is recommended to use only the precipitate obtained by centrifuging and removing the supernatant.

5.4 DNA Extraction

1) Set a small incubator (can be substituted with a thermal cycler) at 98°C and heat it in advance.
2) Add 50 µL of the extraction reagent Solution A to the 1.5 mL tube containing the weighed sample.
3) Heat the 1.5 mL tube containing the sample at 98 °C for 8 minutes.
4) After heating, add 1 mL of extraction reagent Solution B to a 1.5 mL tube at room temperature and mix.
5) Use the supernatant as a DNA extract*.
*The results may be improved by diluting the DNA extract 10 to 50 times.

5.5 Preparation of PCR solution

1) Thaw the PCR mix and primer mix in advance.
2) Prepare a master mix by pipetting and mixing the required number plus 3 samples at a ratio of PCR mix *10 µL and primer mix *5 µL into a 0.2 mL tube for preparing the master mix.

* Mix the PCR mix and primer mix thoroughly by pipetting before dispensing. If the PCR mix shows a white precipitate after thawing, make sure to completely dissolve it before use.

PCR mix	10 µL
Primer mix	5 µL

Example) 5 samples

Required quantity = 5 + 3 = 8 samples

Mix 80 µL of PCR mix and 40 µL of primer mix to make a master mix.

3) Add 5 µL of DNA extract to a new 0.2 mL tube.
4) As a positive control, prepare a 0.2 mL tube containing 5 µL of the positive control DNA included in this kit.
5) As a negative control, prepare a PCR tube containing sterile distilled water or 5 µL of the extract obtained by performing DNA extraction on a blank.
6) Add 15 µL of master mix to each sample and 0.2 mL tube of positive/negative control and mix by pipetting.
7) After adjustment, please keep on ice to prevent enzyme inactivation and non-specific amplification reactions.

5.6 PCR

1) Start the thermal cycler, set the 0.2 mL tube, and then perform the amplification reaction with the following program.

＜**PCR Program**＞

Temp.（℃）	Time（Sec）	Cycle
25	300	1
95	180	1
94	10	35
62	10	
72	40	
72	300	1
10	∞	1

5.7. Test strip detection

1) Remove the 0.2 mL tube after the reaction, open the lid of the 0.2 mL tube, and add 160 µL of chase buffer.

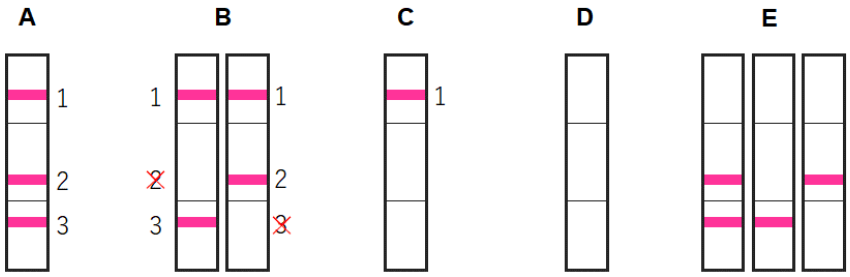
2) Insert the test strip insert into a 0.2 mL tube.

3) After 10 minutes, check the line coloring of the test strip.

【Precautions】

- Determine by line coloring 10 minutes after insertion.
- Wear gloves when handling the test strip and always hold the absorbent pad on the top. Be careful not to touch the test strip insertion part (part with sample) and detection part with your hands.
- When inserting the test strip into the tube, be sure to insert it firmly until the part with the sample hits the bottom of the 0.2 mL tube.
- When inserting the test strip, be careful of contamination due to splashing of the reaction solution.

1： Green plant common line　2, 3： Cannabis detection line



Cannabis	Suspected of Cannabis	Other plants	No plant DNA	Defect
Cannabis positive	**Cannabis negative**			Unable to detect

Pattern A: All three detection lines (1 to 3) are colored

Pattern B: In addition to the green plant common line (1), only one of the cannabis detection lines is colored

Pattern C: Only the green plant common line (1)is colored

Pattern D: No coloring

Pattern E: Only the cannabis detection line is colored, and the common green plant line is not colored.

【Precautions】

- If the positive control does not show pattern A (cannabis positive), the results are invalid because the deterioration of the reagent is suspected.
- Contamination is suspected if the negative control shows anything other than pattern D (no plant DNA). Decontaminate the equipment and the laboratory table and re-inspect.
- If the sample shows pattern E (abnormal), it is suspected that the amount of DNA is insufficient, or impurities impede PCR amplification. Please retest without determining the result.
- Do not draw the final conclusion based on the results of this kit alone but make a comprehensive

judgment together with the results of other detection methods.

6. Notes on using this kit

<Contamination measures>

- To prevent misjudgment due to contamination, replace the used micropipette tip every time. Also, use a micropipette tip with a filter.
- We recommend that each step of DNA extraction, PCR solution preparation, and test strip detection be performed in separate laboratories. If it is difficult to carry out in separate laboratories, separate workbenches, or work areas within the same laboratory.
- Before opening the tubes for reagents, extracted DNA, etc., spin them down with a low speed centrifuge. (To prevent the contents from scattering and reagents attached to the lid from sticking to fingers)
- Regularly clean micropipettes and other equipment and devices used with 0.1% sodium hypochlorite and commercially available DNA removers.
- If contamination occurs, decontaminate with 0.55% sodium hypochlorite (or a commercially available DNA remover) or UV irradiation.

＜Disposal precautions＞

- When discarding the used test strip and amplification solution, be careful not to touch the detection part and put them in a plastic bag, etc. before discarding.
- When discarding the sample after DNA extraction, please take into consideration the sanitary and environmental aspects in accordance with the local regulations on waste and the regulations of the facility.

＜Others＞

- This kit is optimized for Bioer Technology's LifeECO Thermal Cycler. If you are using another model, the result may be unclear.
- Check the equipment and devices used for inspection according to the manufacturer's manual and make sure to use properly operating devices.

7. Bibliography

1) Tadashi Yamamuro *et. al*, Development of simple and accurate detection systems for Cannabis sativa using DNA chromatography, *Forensic Science International*, Volume 291, 2018, 68-75

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