-LAMP Dry Premix-
Cassava Begomovirus (Asian strain)
Universal Detection Kit

Instruction Manual

version 1. 0. 0

Nippon Gene Material

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[Read the following instructions before the test]

Thank you very much for purchasing **-LAMP Dry Premix- Cassava Begomovirus (Asian strain) Universal Detection Kit**. Before using the kit, please confirm the following matters.

Notices for use

1. This kit offers detection of Cassava Begomovirus Asian strain using LAMP, means Loop-mediated Isothermal Amplification. This kit must not be used for clinical diagnosis, therapeutic purpose and the test other than Cassava Begomovirus Asian strain detection.

2. Concerning the storage procedure of the kit, please read section 2 “Notes” for your reference. Every reagent should be kept under proper condition and use it by the expiration date indicated on the product label.

3. Use this kit according to this instruction manual. Nippon Gene Material Co., Ltd. has no responsibility for any trouble caused by the incorrect use and the different purpose from instructions.

4. Concerning the secondary use of the assay result by the kit, please be notified that the user must be responsible for all the consequential damage from the mishandling or misuse. Nippon Gene Material Co., Ltd. has no responsibility for any trouble other than that caused by kit defects.

5. Please avoid running electrophoresis, autoclaving of amplified sample after test and positive control in order to keep the environment free from contaminants.

6. In case of using reagents that are not included in this kit, please follow the notices in the safety instruction of the reagent that you are using. Please do not mix the foreign reagents with the reagents in this kit. Refer to the Safety Data Sheet (SDS) about safe use of this product. If you need the SDS for this product, please contact the contact address on the “Information” section (Last page).

7. This kit is not food. Do not put the reagents into an eye or a mouth. During test, wear a lab coat or gloves and protect the body.

8. Eiken Chemical Co., Ltd. owns the patent right for execution of Loop-mediated Isothermal Amplification. Nippon Gene Material Co., Ltd. has been granted the license to develop, manufacture and sell the kit for Cassava Begomovirus detection.

1. About the kit

Product Overview

Cassava Begomovirus (Asian strain) Universal Detection Kit offers detection of Cassava Begomovirus Asian strain from plant samples using Loop-mediated Isothermal Amplification (LAMP) method. LAMP method is fast and easy DNA amplification method which is also used for the diagnosis of influenza virus and detection of norovirus, *Legionella* sp., *Salmonella* sp., and Verotoxin-producing *E. coli*, exhibiting excellent specificity and sensitivity. In this kit, a part of Cassava Begomovirus DNA amplifies using LAMP method, and infection of Cassava Begomovirus can be judged if the amplification occurs or not.

The operation needed for the detection is extremely easy, simply to mix the DNA extracted from plant sample to the test solution (dissolved C.B.U. Detection Dry Reagent) in a sample tube, and keep the tube at 66 degree Celsius for 60 minutes. The existence of Cassava Begomovirus in the plant can be determined from whether the specific sequence amplifies with LAMP primer set or not.

For detection of DNA amplification, this kit utilizes visual inspection of fluorescence emitted from the solution after the whole reaction, which means that the DNA amplification and detection can be done in one closed tube. Therefore, the amplification of Cassava Begomovirus DNA can be detected safely in a short period of time.

Diagnosis of Cassava Mosaic Diseases

Cassava Begomovirus (Genus Begomovirus, Family Geminiviridae) Asian strain which causes cassava mosaic disease and decrease the yield of cassava, were roughly classified into 2 types of African strain, 1 type of Asian strain.

This kit can detect both Asian strain (such as Sri Lankan cassava mosaic virus (SLCMV) and Indian cassava mosaic virus (ICMV)) universally.

About LAMP (Loop-mediated Isothermal Amplification) Method

LAMP (Loop-mediated Isothermal Amplification) method allows the whole reaction process, including denaturing, to proceed at a constant temperature in an incubator. Thermal cycling machine is not needed for this kit.

Please refer to the homepage of Eiken Chemical Co., Ltd. about the detailed principle of LAMP method.

Eiken GENOME SITE; http://loopamp.eiken.co.jp/e/

2. Reagents provided with the kit

[Kit components (for 48 tests)]

Reagent	Form (top label)	contents (48 tests)	Storage temperature
Instruction Manual	Booklet	1 booklet	-
C.B.U. Detection Dry Reagent	Aluminum bag	8 well x 6 strips	Room temperature
C.B.U. Reagent Dissolve Solution	Orange	400 µl x 6 tubes	
C.B.U. Positive Control	Gray	6 tubes	
C.B.U. Control Dissolve Solution	Green	400 µl x 1 tubes	
Mineral Oil	Blue	400 µl x 6 tubes	

Important

Please test and choose most proper DNA extraction method for your target plant. This kit does not include DNA extraction Kit, so provide it by yourself.

Notes

- Store all reagents in the aluminum bag at room temperature (20-25˚C). Protect them from light. Use by the expiration date indicated on the product label.
  Especially, C.B.U. Detection Dry Reagent and C.B.U. Positive Control should be kept in aluminum bag with desiccant agent to prevent degradation by humidity.

- This kit can perform 6 test reactions when you use 8 well per 1 test. To prevent misjudging derive from contamination of DNA, it is recommended to dispose C.B.U. Reagent Dissolve Solution, C.B.U. Positive Control, Mineral Oil after each test.

- C.B.U. Detection Dry Reagent is dispensed in 8 well strip tube. Please adjust the tube number by cutting the strip with scissors depending on your test size.

- When storing dissolved C.B.U. Positive Control or DNA sample after extraction, please keep it separate from other reagents.

- To prevent misjudging, do not keep dissolved C.B.U. Detection Dry Reagent at room temperature or in a refrigerator for a long time, or freeze it by excessively cold condition.

- C.B.U. Positive Control is the solution of DNA fragment which contains a DNA sequence specific to Cassava Begomovirus genomic DNA. To avoid cross-contamination, do not spill the solution, and avoid the contact of filter tip to the clean equipment and reagents.

- Consecutive dispensing of the reagent may cause cross-contamination, use the filter tip as a disposable in every dispensing batch.

3. Equipment and reagents not provided with the kit

- DNA extraction Kit
- Tweezers (contamination free)
- Micropipette (0.5-10 µl, 10-100 µl, 100-1,000 µl)
- Filtered pipette tip (sterilized)
- Microtube rack
- Aluminum block (or plate rack)
- Vortex mixer
- UV transilluminator (wavelength of 240-260 nm or 350-370 nm)
- An incubator that can maintain 66˚C for a given time
  *Ex. water bath, heating block, thermal cycler, air incubator etc.
- Goggles
- Disposable gloves
- Crushed ice

4. Instructions

[Preparation and precaution before detection]

Sample preparation

■ Control

This kit contains C.B.U. Positive Control to confirm the success or failure of the detection results. To confirm the results are successful, attention should be taken to the preparation of “positive control reaction solution” with C.B.U. Positive Control added, and also the “negative control reaction solution” with DNA elute or dissolve solution added.

■ Sample preparation

Please test and choose most proper DNA extraction method for your target plant. This kit does not include DNA extraction Kit, so provide it by yourself.

Equipment preparation

■ Incubator

Turn on the power of incubator and set the required temperature. In case of using water bath and heat block, it might take time to reach the target temperature so pre-heat the device and confirm the temperature with thermometer. In case of using air incubator, the temperature alters largely when the cabinet door is open. The door opening operation should be quickly done when the sample is set in air incubator.

■ Other equipment

equipment	Instruction
Micropipette	Monopolize micropipette for LAMP working area. Return to the original location after nucleic acid removal operation in case if used in other area.
Microtube rack	Monopolize microtube rack for LAMP working area. Return to the original location after nucleic acid removal operation in case if used in other area.
Filtered pipette tip (sterilized)	Select gamma-ray sterile, nucleic acid-free, nuclease-free grade pipette tip with hydrophobic filter, and unpack at working area. Consecutive

	dispensing of the reagent with one tip may cause cross-contamination, use the filter tip as a disposable in every dispensing batch.
Writing materials	Use solely in each working area, and ensure a dedicated space for the documents brought in the area.
Disposable gloves	Use gloves as disposables, change them when the contamination is suspected.
White robe	Use solely in each working area, be noticed of the contamination from the cuffs.

Testing Environment

Because LAMP method is the DNA amplification technology with excellent sensitivities, it would be difficult to make an accurate inspection if the testing environment is contaminated with C.B.U. positive control or amplified sample after test. For the handling of the sample, take extra care to avoid the contact of the positive control and samples to the working white lobe and equipment. It is also strongly encouraged thorough exchanging of the clothing. To prevent the false results after the test, the used tips, microtubes, and amplified sample after test should be packed together in doubled plastic bags. Please avoid running electrophoresis, autoclaving of amplified sample after test and positive control.

■ Working area

Assign a clean booth or working bench which has not used for nucleic acid extraction and amplification (which has not been contaminated by nucleic acid) as dedicated reagent preparation area. Prepare the test solution only at the reagent preparation area. Do not use C.B.U. positive control, any solution or reagents that may become a template for LAMP method.

Separate the dedicated nucleic acid handling area from reagent preparation area. Addition of sample and C.B.U. positive control must be done only at nucleic acid handling area as the dedicated working area.

■ Nucleic acid decontamination operation

Keep the equipment always clean. Wash the equipment with large amount of tap water to dilute and wash off the nucleic acids on the surface if possible.

If it is suspected to have nucleic acids contamination on the surface of goods, especially after handling highly concentrated nucleic acids, it is recommended to decontaminate the testing environment from nucleic acids with 1% sodium hypochlorite aqueous solution. Sodium hypochlorite generates chlorine gas and corrosive on metals, so it is necessary to wipe immediately the chlorine content from surface when used on metals. Sodium hypochlorite aqueous solution can easily deteriorate under high temperature environment, so take attention of the expiratory date and storage temperature of the solution.

<Protocol for nucleic acid decontamination using 1% sodium hypochlorite aqueous solution>

i) Wear disposable gloves on hands.
ii) Prepare 10,000 ppm (1%) sodium hypochlorite aqueous solution.
iii) Gently wipe the working bench and equipment with paper towel moistened with sodium hypochlorite aqueous solution followed by wiping with paper towel moistened with 70% ethanol.
iv) For non-metal equipment, soak the equipment in sodium hypochlorite aqueous solution for more than one hour followed by rigorous rinsing with water and drying.
v) Keep working bench and equipment always clean, and perform wiping by sodium hypochlorite aqueous solution regularly.

[Detailed protocol]

To avoid template DNA contamination, please separate **"nucleic acid handling (step A, B) area"** and **"reagent preparation (step C) area"** for testing.

Detailed Protocol

* Please do not proceed all procedure at the same time,
  carry out each process in order of A → B → C.
* Please wear disposable gloves during step A to C and change them when you go next step.

<A. preparation of DNA sample from plant tissue>

A-1. Extract DNA from plant tissue and use it as "**DNA Sample**" for LAMP reaction.

Important

*"Collection part from plant tissue" and "DNA extraction method (Ex. commercialized DNA extraction kit, CTAB method)" should be tested and optimized by yourself.
*DNA elute or dissolve solution is "**Negative Control**".

<B. Preparation of C.B.U. Positive Control Solution>

B-1. Take out one of **C.B.U. Positive Control** tube.

* To avoid absorption of moisture, the remaining tubes should be sealed in aluminum bag immediately.

B-2. Add 10 µl of **C.B.U. Control Dissolve Solution** in above tube.

B-3. After spinning down, leave it at room temperature for 5 minutes.

* Because the dried **C.B.U. Positive Control** (red color reagent) exists in the bottom of tube, make sure to contact the control with **C.B.U. Positive Control Dissolve Solution** completely.

B-4. After vortexing, spin down the tubes (**C.B.U. Positive Control Solution**).

<C. Preparation of test solution and LAMP reaction>

C-1. Take out the required number of **C.B.U. Detection Dry Reagent** by tweezer.

* The tubes of required number (sample number and control number) must be kept on ice (using aluminum rack or plate rack) .
* To avoid absorption of moisture, the remaining tubes should be sealed in aluminum bag immediately.
* If the dry reagent is attached on lid, shake the tube gently and pull down the reagent at bottom of tube.

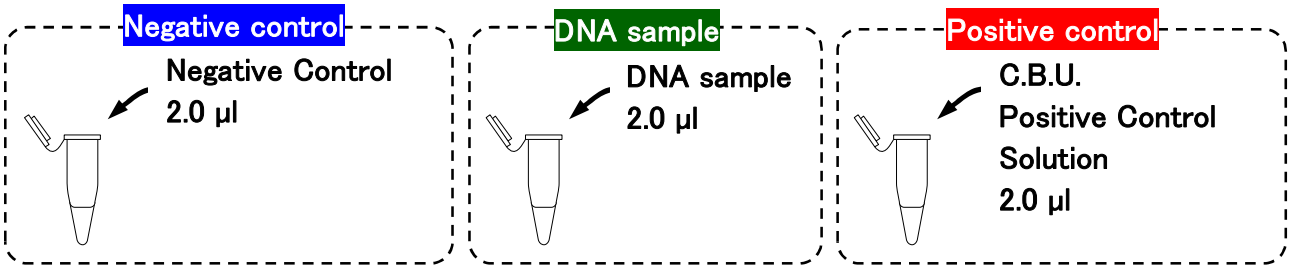
C-2. Take out C.B.U. Reagent Dissolve Solution and Mineral Oil.

* Tap or vortex for 1 second x3 times, then spin down.

C-3. Add 23 µl of **C.B.U. Reagent Dissolve Solution** to each tube.

C-4. Add 2 µl of sample, close the cap, and spin down.

* When adding the sample, be sure to operate with the following order.
Add 2.0 µl of **Negative Control** to negative control tube and close cap. Then add 2.0 µl of **DNA Sample** to sample reaction tube and close cap. Finally, add 2.0 µl of **C.B.U. Positive Control Solution** to a positive control tube and close cap.
* After closed cap, remove the air by spin down and make sure that they contact with **C.B.U. Detection Dry Reagent** and **C.B.U. Reagent Dissolve Solution**.



C-5. After 2 minutes, overlay with 20 µl of **Mineral Oil**.

* If dry reagent and **Mineral Oil** come into contact, it may cause insoluble precipitation.
If you use **Mineral Oil**, make sure to add it after dry reagent are completely dissolved.

C-6. Invert 5 times to mix, then spin down.

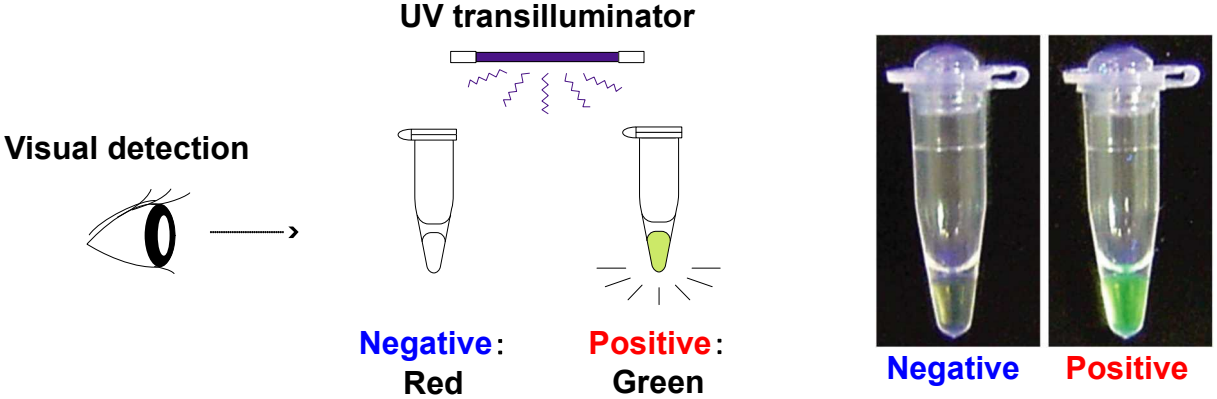
* When mixing the solution, do not use vortex mixer to avoid bubbles.

C-7. Incubate test tube at 66˚C for 60 minutes (test reaction).

C-8. Terminate the reaction at 80˚C for 2 minutes.

C-9. Judgement

* Check the change of color. If positive, the test solution changes to a vivid yellow-green color, and if negative, it remains a pale red color. If you confirm the change of color, it indicates the possibility of the existence of a target in this sample. That color derives from fluorescence so that you can get more precise judgment under UV light. For fluorescence detection, UV lamp device (wavelength: 240-260 nm or 350-370 nm) and goggles or face shields are required. If you are using controls, please confirm that the positive control test solution turns blight yellow-green color, and negative control test solution remains pale red. If the result does not meet the conditions, invalidate the result, and investigate the cause.



5. Troubleshooting

If you experience trouble with this kit, check the items on below and try the solutions. Consult Nippon Gene Material Co. Ltd. for further questions.

Problem	Possible cause and solution
Control test solution does not give the right coloring.	**A. Too much C.B.U. Positive Control added to the test solution** There are some cases that efficiency of test reaction decreases when too much C.B.U. Positive Control is added to the reaction. Please follow the instruction for the correct amount of addition. **B. Reagents or testing environment are contaminated with nucleic acid** In case of negative control testing solution gives coloring, template DNA contamination is suspected. Contamination monitoring of reagents and testing environment, cleaning procedure by 1% sodium hypochlorite aqueous solution are recommended to remove completely the contaminants. After the removal, redo the test. **C. Chelate compounds or metal ion in the sample** Test Solution emits fluorescence when chelate compounds such as EDTA exists in the reaction. On the other hands, if a lot of metal ion presents in reaction, the fluorescence is inhibited thus it would be difficult to judge the result. **D. Reaction temperature and operating procedure not correct** Confirm that there is no problem on the test process.
Irregular coloring of test solution	**A. Judgement not immediately after test reaction has ended** Test Solution irregularly gains or loses its coloring when left at room temperature for long time. Please judge immediately after LAMP reaction finished.
Test solution has evaporated.	**A. The reaction tube not heated homogeneously** Water bath, heat block may have not heated the test tube homogeneously so that the test solution would be concentrated because of evaporation. In such case the reactivity efficiency goes down. Make sure that mineral oil to be added to the test solution.
The judgement of fluorescence is difficult.	**A. UV lamp wavelength not optimal.** UV lamp emitting light wavelength of 240-260 nm or 350-370 nm is necessary for the detection. In case of the wavelength of the light is 320 nm, be notified that negative sample could emit fluorescence (false-negative).
There are not enough reagents for testing.	**A. Reagent sticks on the inner tube surface.** Spin down the microtube before use. **B. Reagent evaporated during its storage** Completely close the cap after use.

6. Reference

1. Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T. (2000) Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res*. **28** (12): e63

2. Prince AM, Andrus L. (1992) PCR: how to kill unwanted DNA. *Biotechniques*. **12** (3): 358

[Memo]

- It may change without a preliminary announcement about the written contents of the description, product specification, and a price.
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