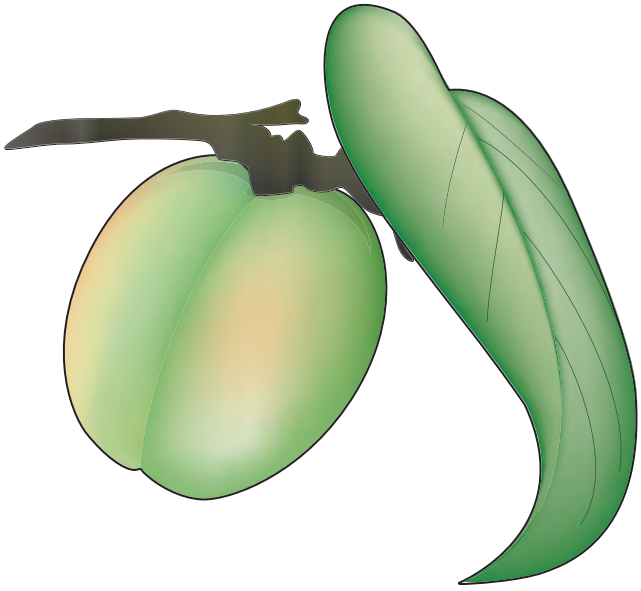
plum pox virus Detection Kit

Instruction Manual

version 1.0



NIPPON GENE CO., LTD.

plum pox virus Detection Kit

Instruction Manual Version 1. 0

[Read the following instructions before the test]

Thank you very much for purchasing **plum pox virus Detection Kit**. Before use the kit, please confirm the following matters.

Notices for use

1. This kit is the product to detect plum pox virus (PPV). This kit must not be used for clinical diagnosis, therapeutic purpose and the test other than PPV detection.

2. Use the kit according to this instruction manual. Nippon Gene Co., Ltd. and the distributors of this kit have no responsibility for any trouble caused by the different use and the different purpose from instructions.

3. The unopened kit is stable at -20˚C for 6 months. It should be kept in dark. Avoid repeated freezing and thawing.

4. plum pox virus Detection Kit is licensed from Eiken Chemical Co., Ltd. Nippon Gene Co., Ltd. has been granted the license to development, manufacture and sell the kit for plum pox virus identification.

5. Refer to the Material Safety Data Sheet (MSDS) about safe use of this product. MSDS is exhibited in the homepage of Nippon Gene Co., Ltd.
   URL; **http://nippongene-analysis.com/e/**

6. 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.

Table of contents

1. About the kit

[Product overview]

This kit is the product to detect plum pox virus (PPV) by reverse transcription and isothermal amplification reaction using LAMP (Loop-mediated Isothermal Amplification) method. This product can test whether PPV exists in a Japanese apricot (*Prunus mume*). In this kit, a part of PPV genomic RNA is amplified using LAMP method, and PPV infection is judged by the existence of amplification. This kit is effective for the highly-sensitive detection of PPV. It was developed by the Plant Clinic of the University of Tokyo.

The Plant Clinic of the University of Tokyo detected first PPV-D from the Japanese apricot (*Prunus mume*) in Japan March 2009. And then, they developed the LAMP method to detect the PPV from results of the gene analysis of PPV-D which was detected in Japan.

[About LAMP method]

LAMP (Loop-mediated isothermal amplification) method allows the whole reaction process, including denaturating, proceeds at a constant temperature in a incubator. Thermal cycling machine is not needed for this kit.
Look at 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)]

Form	Reagent	Contents	Storage temperature
Booklet	**Instruction Manual**	**1 sheet**	Room temperature
Red label	**PPV Detection Solution**	**1,150 µl**	-20 ˚C
Yellow label	**Enzyme Solution**	**50 µl**	-20 ˚C
Purple label	**Fluorescent Detection Solution**	**50 µl**	-20 ˚C
Gray label	**PPV Positive Control**	**25 µl**	-20 ˚C
Blue label	**Mineral Oil**	**1,000 µl**	-20 ˚C
Bag	**Test Tube**	**48 tubes**	Room temperature

Notes

Store all reagents other than **Instruction Manual** and **Test Tube** at -20˚C. Avoid storage in extremely cold environments, and avoid repeated freezing and thawing.
This kit can be used for 6 months after purchase.
The **Enzyme Solution** must be handled with great care. Do not leave it at room temperatures or 4˚C for a long period of time, or the enzyme may loose activity.
PPV Positive Control is the RNA that contains a fragment of PPV genomic RNA.
When waterdrop is attached to the **Test Tube**, dry a bag well before opening.

Positive Control

Always run positive control with the sample. To prepare **Positive Control Solution**, add the provided **Positive Control** (Gray label) instead of the sample.

Negative Control

Always run negative control with the sample. To prepare **Negative Control Solution**, add the sterilized distilled water or add nothing instead of the sample.

3. Equipments and reagents not provided with the kit

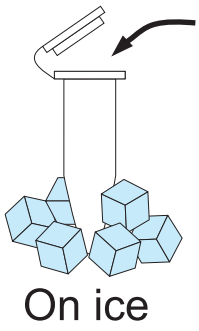
- Micropipette
  (0.5 - 10 µl, 10 - 100 µl, 100 - 1,000 µl)
- Filtered pipette tip (sterilized)
- Disposable glove
- Sterilized microtube for preparation of **Test Solution** (0.5 ml or 1.5 ml)
- UV goggles or face shield
- Crushed ice and ice box
- Wood toothpick
- Incubator which can keep it warm at 60 - 65 ˚C
- Microtube rack
- Plate rack
- Vortex mixer
- Centrifuge for microtube
- Centrifuge for test tube
- UV transilluminator which outputs the wavelength of 254 - 366 nm

4. Instructions

[Simple protocol]

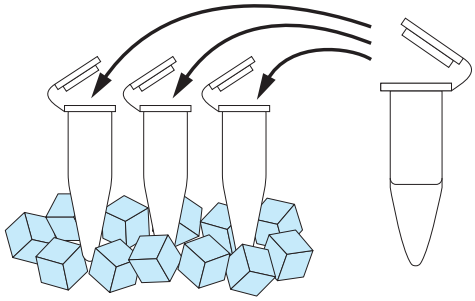
Simple protocol

1 Prepare Test Solution

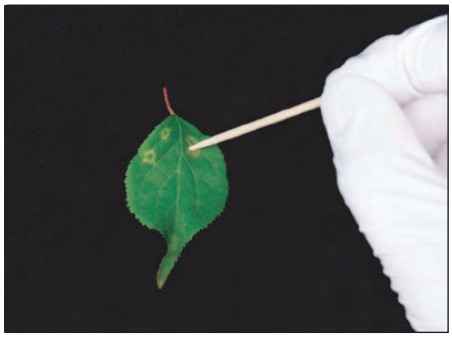


	1 test	8 test	24 test
PPV Detection Solution	23 µl	184 µl	552 µl
Fluorescent Detection Solution	1 µl	8 µl	24 µl
Enzyme Solution	1 µl	8 µl	24 µl
Total	25 µl	200 µl	600 µl

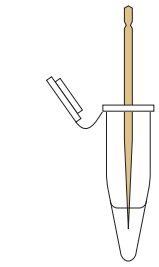
2 Divide Test Solution into each Test Tube



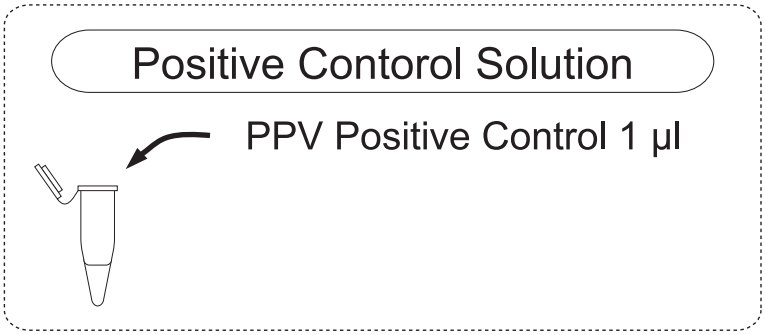
3 Prick the ringspot of leaf with a toothpick



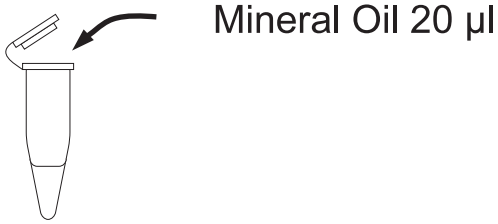
4	**Dip the toothpick in Test Solution**



Test Solution



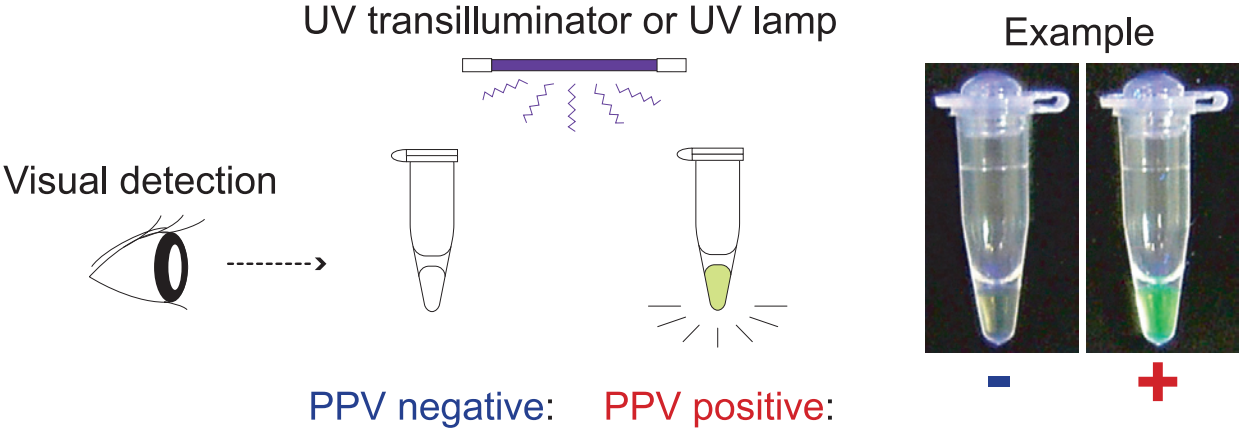
5	**Add Mineral Oil**



6	**63°C, 1 hours (Nucleic acids amplification)**

7	**80°C, 2 minutes (Enzyme inactivation)**

8	**Judge the result**



Preparation

[Preparation of the sample]

Control

Be sure to prepare **Positive Control Solution** and **Negative Control Solution** for every test. To prepare **Negative Control Solution**, add the sterilized water or nothing instead of the sample.

Preparation of the sample

Each toothpick which you have pricked the leaves should be put into a clean plastic bag to avoid contamination.

It is also possible to test by collecting some portions of a Japanese apricot or other plants (a leaf or stalk). Moreover, the virus may be infected even if the leaf of a Japanese apricot has not presented signs. Observe a leaf well and test a doubtful leaf.

After the test, dispose the tubes according to the relevant regulations and instructions. To prevent the amplified products from dispersing, do not conduct autoclave sterilization treatment for disposal.

[Preparation of equipments]

Incubator

Warm an air incubator, a heating block, or a water bath at 63˚C before beginning test.

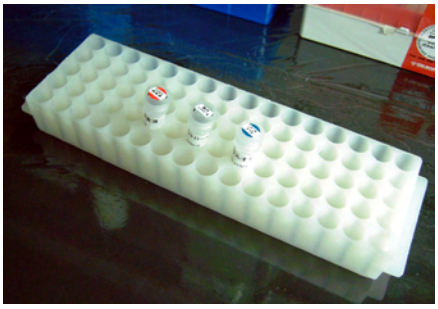
Laboratory

Since LAMP method shows very high sensitivity, it becomes difficult to obtain exact result if there is contaminations of plum pox virus or **PPV Positive Control** in the room. Take care of handling the plum pox virus contained sample, **PPV Positive Control** and the amplified product.

PPV diagnosis

1. Test reaction

1-1. Thawing of the reagents

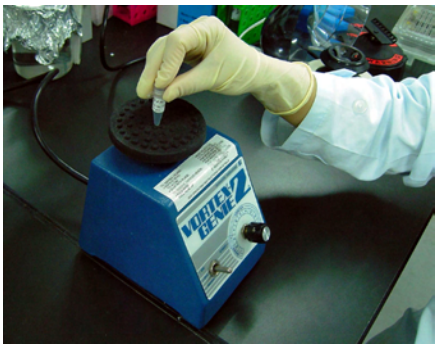


Thaw the following reagents at room temperature.

PPV Detection Solution
PPV Positive Control
Mineral Oil

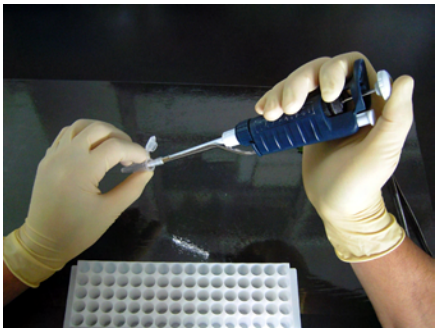
Enzyme Solution and **Fluorescent Detection Solution** do not freeze at -20˚C.

1-2. Mix and spin down



Gently tap the tubes several times (This operation is called tapping.), or vibrate the tubes 3 times for approximately 1 second on a vortex mixer. After the mixture becomes homogenous, centrifuge the tubes to collect the solution at the bottom of the tubes (This operation is called spin down.).
The reagents, **Enzyme Solution** and **Fluorescent Detection Solution**, are placed on ice.

1-3. Preparation of **Test Solution**

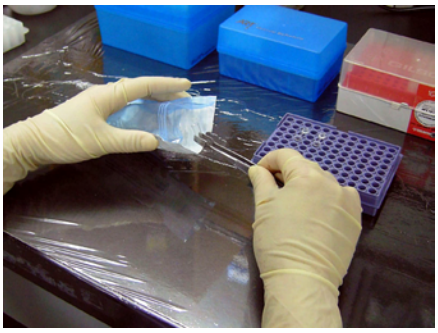


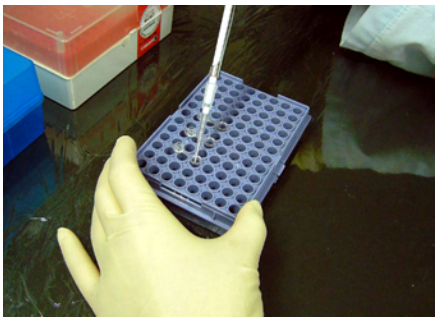
To make **Test Solution**, add **PPV Detection Solution**, **Fluorescent Detection Solution**, and **Enzyme Solution** to a sterilized microtube (0.5 ml or 1.5 ml). Mix the reagents by tapping or vibrating 3 times for approximately 1 second on a vortex mixer, and spin down the tube. It places on ice.
Refer also to the following table.

[Table of volume]

Reagent	**1 test**	8 tests	24 tests
PPV Detection Solution	**23 µl**	184 µl	552 µl
Fluorescent Detection Solution	**1 µl**	8 µl	24 µl
Enzyme Solution	**1 µl**	8 µl	24 µl
Total	**25 µl**	200 µl	600 µl

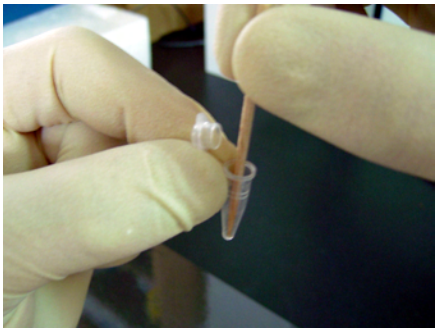
1-4. Distribution of **Test Solution**



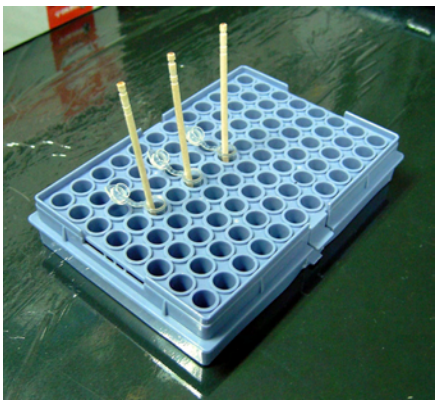


Pick a tube out with clean tweezers, etc. from a bag. Divide 25 µl of **Test Solution** into each **Test Tube**.

1-5. Addition of the sample



The toothpick which you pricked the leaf, is dipped into **Test Solution**. After all samples are added, 1 µl of **PPV Positive Control** will be added to **Test Tube** of **Positive Control Solution**. To prepare **Negative Control Solution**, add sterilized distilled water or nothing instead of sample. Then 20 µl of **Mineral Oil** is added to all **Test Tube**.

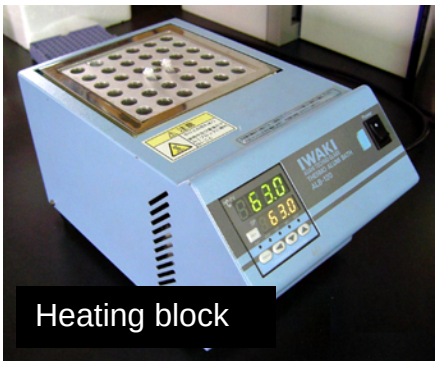


Important

- Do not leave the toothpick in **Test Solution**. The volume of detection mixture is reduced because the toothpick absorbs **Test Solution**.
- **Mineral Oil** is essential to prevent evaporation of **Test Solution**, which may cause a false positive.

1-6. Incubation

Air incubator

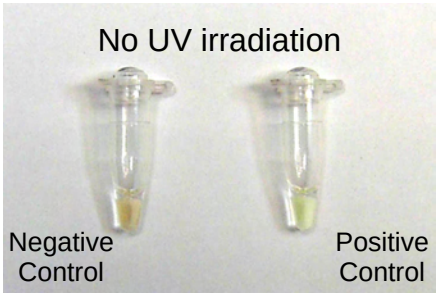


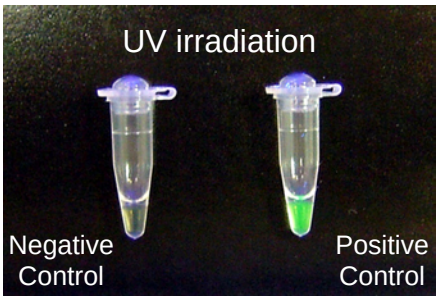
Heating block

Close **Test Tube** and mix the reagents by tapping or vibrating on a vortex mixer at about 1 second x 3 times, and spin down the tube.
Incubate **Test Tube** at 63˚C for 1 hour in the incubator (Air incubator, heating block, or water bath.).

2. Judgment

2-1. Judgment of success or failure of the test





After 1 hour passes, terminate the reaction by heating for 2 minutes at 80˚C.
Fluorescence Detection Solution before reaction looks light red. If the virus exists, it turns to vivid yellow under UV irradiation.
In this procedure, UV transilluminator (wavelength: 254 - 366 nm), UV goggles, or a face shield is needed.
Normally, **Positive Control Solution** appears fluorescence and **Negative Control Solution** does not appear fluorescence. If **Negative Control Solution** appears fluorescence, there is possibility that **PPV Positive Control** enters the test tube by mistake. In this case, check the handling and try to test again.

Important

Judge the result immediately after the reaction is completed.

2-2. Judgment of the sample

[Judgment]

If **Test Solution** appears the fluorescence that is same level with the **Positive Control Solution** under UV irradiation, the sample may contain PPV. If the fluorescence is NOT detected under UV irradiation, the sample does not contain PPV.

[The tips of judgment]

Test Solution has emitted fluorescence clearly

The plant may be infected with PPV.

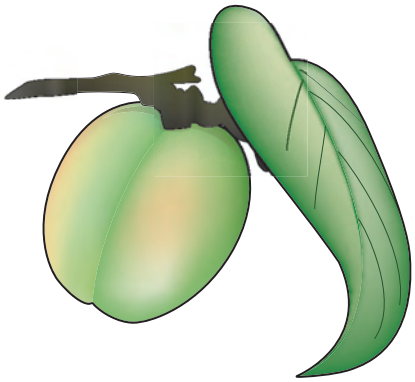
Test Solution does not have any difference in fluorescence as compared with **Negative Control Solution**

The plant may not be infected with PPV. However, fluorescence may not appear in the early stage of infection, since virus concentration is very low. Even if the plant has not presented symptoms, test again after ten days when the infection is suspected.

5. References

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



Information

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