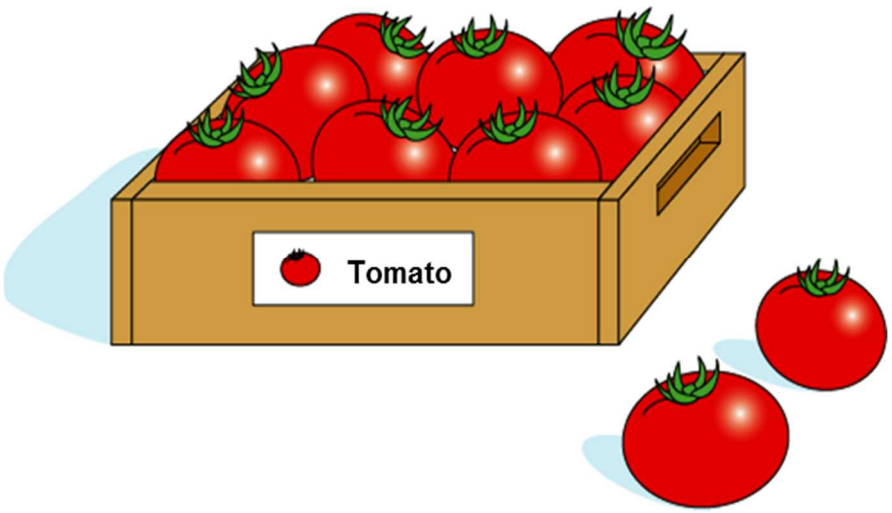
TYLCV Detection Kit Ver.2

Instruction Manual
version 11.0.1



NIPPON GENE

TYLCV Detection Kit Ver.2

Instruction Manual version 11. 0. 1

[Read the following instructions before the test]

Thank you very much for purchasing **TYLCV Detection Kit Ver.2**. Before using the kit, please confirm the following matters.

Notices for use

- This kit is the product to detect TYLCV (*Tomato yellow curl leaf virus*) using LAMP method. This kit must not be used for clinical diagnosis, therapeutic purpose and the test other than TYLCV detection.

- Concerning the storage procedure of the kit, please read section 2 “Notes” for your reference. The unopened kit is stable at -20˚C for 6 months. It should be kept in dark. Avoid repeated freezing and thawing.

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

- 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 Co., Ltd. has no responsibility for any trouble other than that caused by kit defects.

- Regular test is needed because there is a case virus is not detected depend on the infected part, infection level, and virus concentration. This kit is a simple detection kit for auxiliary testing, so definite diagnosis cannot be guaranteed.

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

- 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. SDS is exhibited in the homepage of Nippon Gene Co., Ltd.
  URL; https://nippongene-analysis.com/

- 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.

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

Table of contents

1. About the kit

Product Overview

TYLCV Detection Kit offers detection of TYLCV (TYLCV; *Tomato yellow leaf curl virus*) from plant samples or silverleaf whitefly using Loop-mediated Isothermal Amplification (LAMP) method. LAMP method is a 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 TYLCV DNA amplifies using LAMP method, and infection of TYLCV can be judged if the amplification occurs or not.

The operation needed for the detection is extremely easy; simply soak the tooth pick into the test solution (mixture of TYLCV Detection Solution, TYLCV Enzyme Solution, and Fluorescent Detection Solution) in a sample tube after pricking tomato leaf, other plant sample or silverleaf whitefly, and keep the tube at 60-65 degree Celsius for 60 minutes. The existence of TYLCV in the sample can be judged from whether the specific sequence amplified 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 TYLCV DNA can be detected safely in a short period of time.

Diagnosis of TYLCV

Tomato yellow leaf curl disease is caused by infection of TYLCV which has arisen in Japan since the middle of 1990s. TTLCV is divided into Geminiviridae family, begomovirus genus and has cyclic single stranded DNA as genomic DNA. TYLCV infection to tomato causes reduction of yields because of insufficient growth subsequently to chlorosis and dwarf of leaves. Today, it is difficult to prevent the extension of virus infection completely since silverleaf whitefly, the vector, is such minute that discovery and destruction are troublesome nevertheless various countermeasures against TYLCV has been taken. Furthermore, since 2009, silverleaf whitefly-biotype Q has been spreading which has different pesticide resistance to silverleaf whitefly-biotype B, the conventional vector, which makes the situation much more difficult.

Even though ELISA (Enzyme-Linked Immunosorbent Assay) and PCR (Polymerase Chain Reaction) has been developed to detect TYLCV, immuno-chromatography such as ELISA needs antiserum and antibody, furthermore, its low sensitivity leaves a problem of false positive/negative. Presently, DNA amplification by PCR is considered to be valid way to detect TYLCV, but it needs at least two steps of repeated temperature change and needs electrophoresis to consult the result, which matters when applying such troublesome method into diagnostic area.

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

Eiken GENOME SITE; https://loopamp.eiken.co.jp/en/

About The Synthetic Oligonucleotide Included in This Kit

The primers included in this kit are all “Reliable & Traceable oligo”. “Reliable & Traceable oligo” is one of the highly reliable oligonucleotide series manufactured under control of ISO 13485:2003 certification by Nippon Gene Material Co., Ltd. The oligonucleotides are all produced under dedicated positive pressure environment, with checklists to control process and ensure the full traceability of the production.

Please refer the homepage of Nippon Gene Material Co., Ltd. about the details of “Reliable & Traceable oligo”.

Nippon Gene Material Co., Ltd.; https://www.nippongenematerial.com/

2. Reagents provided with the kit

[Kit components (for 10 tests)]

Reagent (tube label)	Form (top label)	Contents 10 tests	Storage temperature
Instruction Manual	Booklet	1 booklet	Room temperature
Test Tube	Bag	10 tubes	Room temperature
TYLCV Detection Solution	Red label	230.0 µl	-20˚C (avoid light)
TYLCV Enzyme Solution	Yellow label	15.0 µl	-20˚C (avoid light)
Fluorescent Detection Solution	Purple label	15.0 µl	-20˚C (avoid light)
TYLCV Positive Control	Gray label	5.0 µl	-20˚C (avoid light)
Mineral Oil	Blue label	400.0 µl	-20˚C (avoid light)

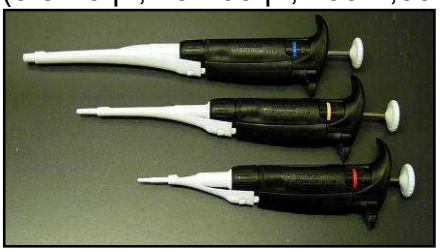
Notes

- This kit can set up the test reactions up to 10 samples by preparing test solution for 10 tests in one tube. Please be noticed that the number of the test that you can do will be less than 10 if you prepare the test solution over several times from one kit.
- If the water drop is attached to the test tube or bag, completely dry it before you open and use.
- Store all reagents other than Instruction Manual and Test tube at -20˚C. Protect them from light. Use until 6 months upon arrival date.
- The reagents should be thawed each time when used, and the remaining should be stored at -20˚C again. Repeated freeze-thaw may deteriorate the quality of this kit. Aliquot the contents to several tubes if necessary.
- TYLCV Enzyme Solution must be handled with extra care. Do not leave the solution at room temperature or 4˚C for a long period of time. Do not freeze it. Under inappropriate temperature condition, the enzyme may lose its activity.
- TYLCV Positive Control is the solution of DNA fragment which contains a DNA sequence specific to TYLCV genomic DNA. To avoid cross-contamination, do not spill the solution, and avoid the contact of the used 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.
- Mineral Oil might belong to the hazardous substance depending on the local administration. Please refer to the local regulation concerning hazardous substances.

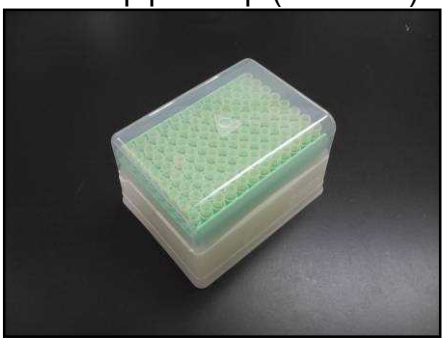
3. Equipment and reagents not provided with the kit

[Required equipment and reagent]

- Micropipette
  (0.5-10 µl, 10-100 µl, 200-1,000 µl)



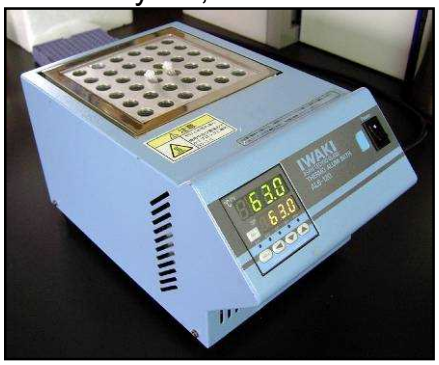
- Filtered pipette tip (sterilized)



- Microtube for preparation of test solution
  (1.5 ml or 2.0 ml)

- Disposable gloves

- Incubator
  Any equipment that can maintain 60-65˚C for given time (e.g. water bath, heating block, thermal cycler, air incubator etc.)

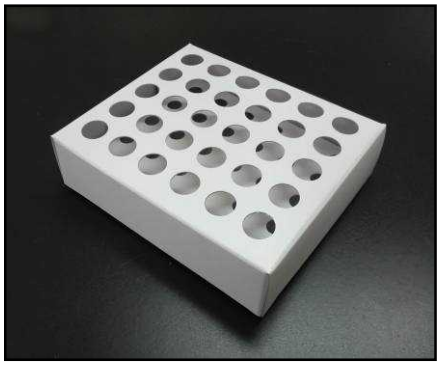


- Tooth pick made of wood

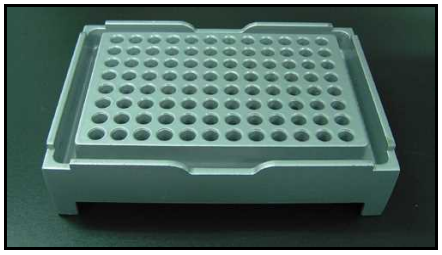
[Equipment which may be useful]

Use these equipment if necessary:

- Microtube rack



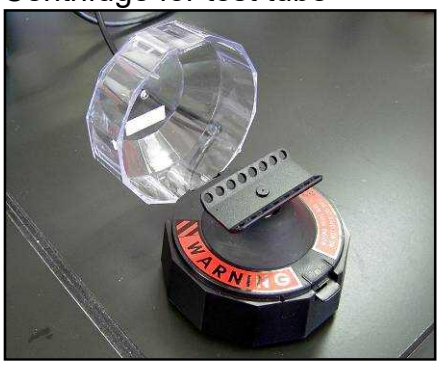
- Plate rack



- Vortex mixer

- Centrifuge for microtube

- Centrifuge for test tube



- Float plate
  Use when the sample needs to be heated with water bath.

- UV transilluminator
  Use when the sample is detected with Fluorescent detection solution.
  Transilluminator should output the light with wavelength of 240-260 nm or 350-370 nm.

- UV goggles or face shield
- Tweezers
- Crushed ice
- Vinyl pack for sampling
- 100 mM Tris-HCl (pH 8.0)
- 0.5 N Sodium hydroxide

4. Instructions

[Simple protocol]

For the detailed protocol of this kit, please refer to the instruction at page 7 and later.

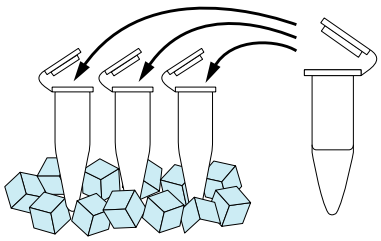
Simplified Protocol

1. Prepare the test solution

Reagent	1test	4+1test *	8+1test*
TYLCV Detection Solution	23.0 µl	115.0 µl	207.0 µl
Fluorescent Detection Solution	1.0 µl	5.0 µl	9.0 µl
TYLCV Enzyme Solution	1.0 µl	5.0 µl	9.0 µl
Total	25.0 µl	125.0 µl	225.0 µl

* Prepare the test solution for the number of the samples that you test + 1 test, so that you have enough volume for the test solution of all samples.

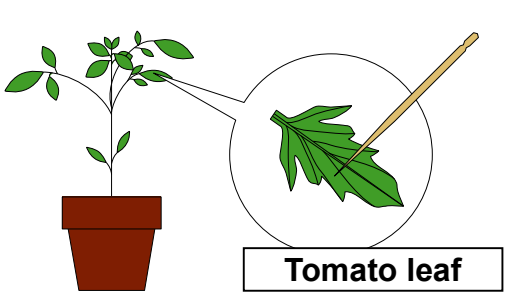
2. Transfer 23.0 µl aliquot of the test solution into the test tube

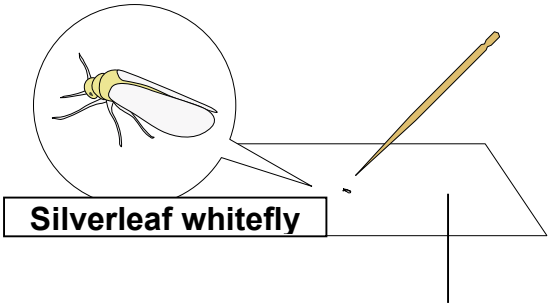


test solution 25.0 µl

On ice

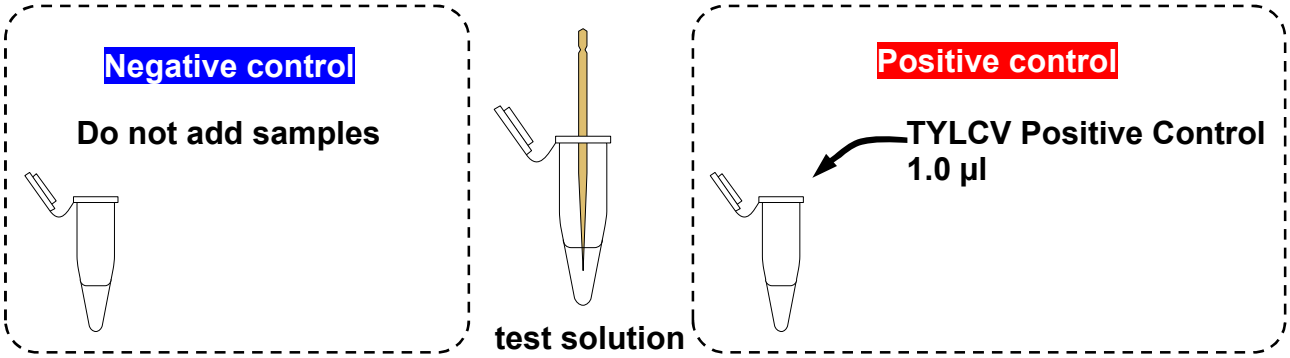
3. Prick the sample by tooth pick



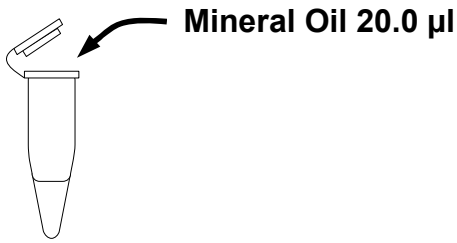


Plastic wrap or paraffin film

4. Soak the tooth pick into test solution



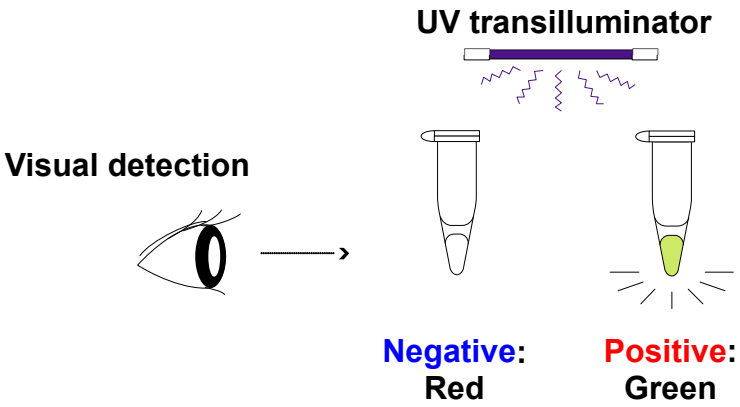
5. Add 20 µl of Mineral Oil

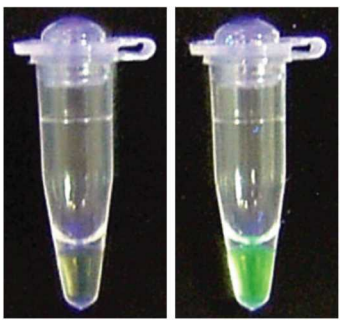


6. Incubate at 63˚C for 60 minutes (DNA amplification)

7. Incubate at 80˚C for 2 minutes (Enzyme inactivation)

8. Judge the result





[Preparation and precaution before detection]

Sample preparation

■ Control

This kit contains TYLCV Positive Control to confirm the success or failure of the detection results. To confirm that the results are successful, attention should be paid to the preparation of “positive control reaction solution” with TYLCV Positive Control added, and also the “negative control reaction solution”.

■ Sample preparation

In case of using this kit in the field, prick a part of tomato or other plants (in case of tomato, pick up the terminal bud or second leaf from the top) which is suspected to be infected with TYLCV and put the tooth pick in a plastic bag. In order to prevent contamination and false positive/negative, keep the tooth pick separated each other, and when collecting samples, wear disposable groves so that they can be disposed when touched by the edge of the tooth pick. Sample is on the edge of the tooth pick and can be used for detection directly.

Detection with tooth pick can be done also by using samples from tomato or other plant’s leaf or stem after collecting and putting into plastic bag. Although withered samples can be used, please use fresh samples for stable results. Observe the leaves carefully and test from the most suspicious one because even if there is no symptom, TYLCV can exist. Furthermore, virus can be detected from TYLCV resistant varieties with no symptom.

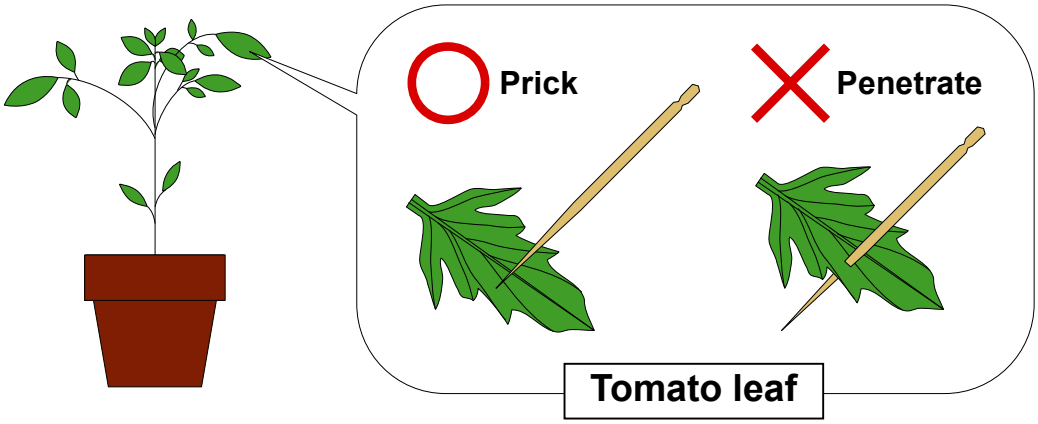
When using silverleaf whitefly as a sample, catch one in a container which can be made airtight, add appropriate amount of 100 mM Tris-HCl (pH 8.0) or sterilized water in order to prevent dispersion, and then prick the sample using tooth pick. It is also possible to test with samples attached on yellow adhesion board by pricking using tooth pick, however, please note that the test solution can be coloring green regardless of TYLCV existence if too much adhesion liquid is added.

In sample preparation, appropriate measures in each facility should be performed in order not to diffuse TYLCV in other cultivation environment. Especially, be careful not to transport silverleaf whiteflies between facilities by using insect proof net and insecticide, and by changing clothes. In order to prevent false positive/negative, used tooth picks and vinyl packs, and samples after the test should be in doubled plastic bag and dispose. Furthermore, please avoid autoclaving of disposed samples.

[How to prick the leaf]

How to prick the leaf

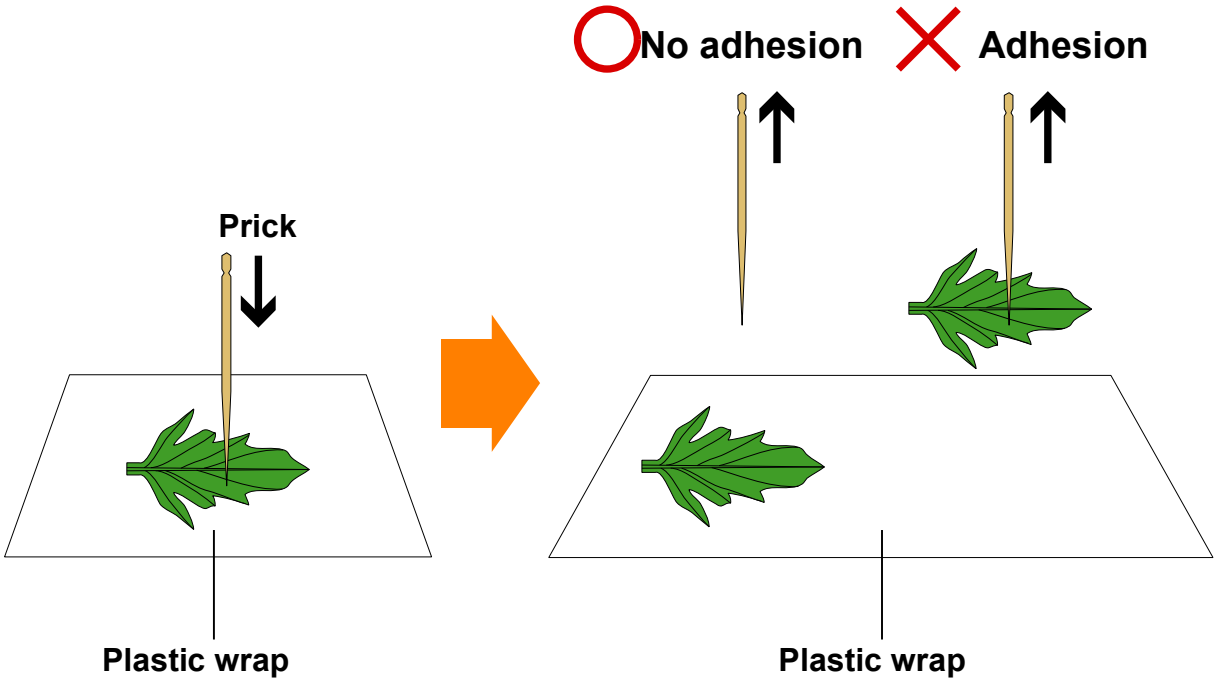
When you prick a sample, do not penetrate the leaf. Too much juice of the leaf may cause false positive.



In case if false positive is suspected, retest by pricking the leaf lightly on the hard table with plastic wrap as following.

Important

In case that the leaf adheres to the tooth pick after picking, you should have picked more lightly.



[Preparation and precaution before detection]

Equipment preparation

■ Incubator

Turn on the power of an incubator and set the required temperature. In case of using a water bath and a heat block, it might take time to reach the target temperature, so pre-heat the device and confirm the temperature with a thermometer. In case of using an 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 an air incubator.

■ Other equipment

equipment	Instruction
Micropipette	Use micropipette exclusively in LAMP working area. Return to the original location after nucleic acid removal operation in case if used in other area.
Microtube rack	Use microtube rack exclusively in LAMP working area. Return to the original location after nucleic acid removal operation in case if used in other area.
Microtube	Select gamma-ray sterile, nucleic acid-free, and nuclease-free grade microtube.
Filtered pipette tip (sterilized)	Select gamma-ray sterile, nucleic acid-free, and 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 expendables 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 expendables, 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 TYLCV 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. Exchange of clothes is also strongly recommended. 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 with nucleic acid) as dedicated reagent preparation area. Prepare the test solution only at the reagent preparation area. Do not use TYLCV 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 TYLCV 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 is 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 pay attention to the days elapsed and storage temperature since 1% solution has been prepared.

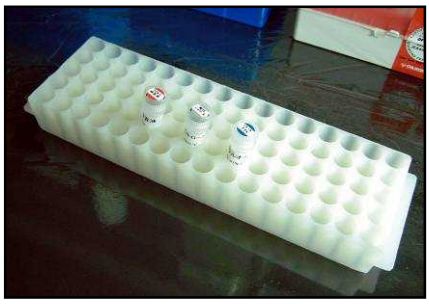
<Protocol for nucleic acid decontamination using 1% sodium hypochlorite aqueous solution>

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 a paper towel moistened with sodium hypochlorite aqueous solution followed by wiping with a 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]

Test reaction

Thawing of the reagents



Thaw the following reagents at room temperature.
TYLCV Detection Solution
TYLCV Positive Control
Mineral Oil
Take out TYLCV Enzyme Solution and Fluorescent Detection Solution from the freezer just before use because they do not freeze at -20˚C.

Mix and spin down

Gently tap the tubes several times (hereinafter referred to as “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 in the bottom of the tubes (hereinafter referred to as “spin down”), and then put the reagents on ice.

Preparation of Test Solution

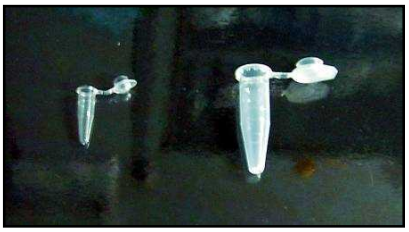
To make Test Solution, add TYLCV Detection Solution, Fluorescent Detection Solution, and TYLCV Enzyme Solution to a sterilized microtube (1.5 ml or 2.0 ml). Mix the reagents by tapping or vibrating 3 times for approximately 1 second on a vortex mixer, and spin down the tube. Place the tube on ice.

Refer also to the following table.

[Table of volume]

Reagent	1test	4+1 tests	8+1 tests*
TYLCV Detection Solution	23.0 µl	115.0 µl	207.0 µl
Fluorescent Detection Solution	1.0 µl	5.0 µl	9.0 µl
TYLCV Enzyme Solution	1.0 µl	5.0 µl	9.0 µl
Test Solution Total	25.0 µl	125.0 µl	225.0 µl

* Prepare the test solution for the number of the samples that you test + 1 test, so that you have enough volume for the test solution of all samples.

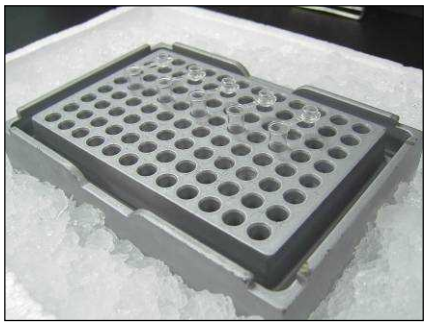


Important

For preparation of test solution, use another 1.5 ml microtube (right side one of the photograph), and do not use the test tubes attached. Prepare the test solution for the number of the samples that you test + 1 test, so that you have enough volume for the test solution of all samples. Consecutive dispensing of the reagent may cause cross-contamination, use the filtered tip as a disposable in every dispensing batch.

TYLCV Enzyme Solution is extremely viscous so be careful not to stick too much amount of solution on the outer surface of the filtered micro tip. Spin down prior to use.

Distribution of Test Solution

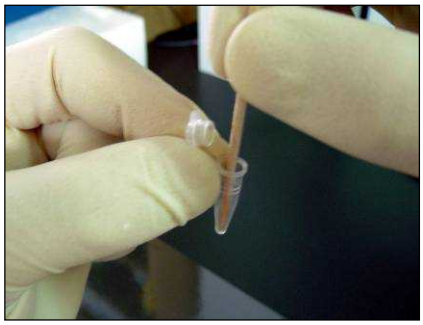


Pick a Test Tube out from the bag with a clean tweezer. Dispense 23.0 µl of Test Solution into each Test Tube.

Important

Only use the specified Test Tube which is attached to this kit. Use of different volume, shape, and material may cause misjudgment.

Sample addition



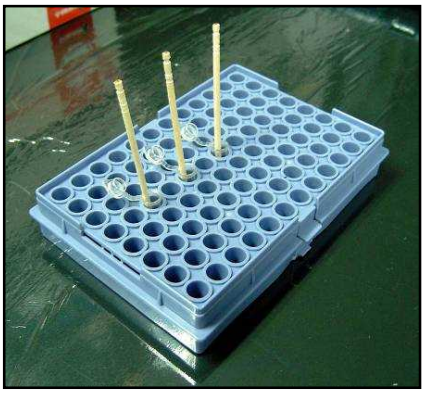
Soak the tooth pick into the test solution after pricking the sample, and rub the bottom of the tube lightly. After that immediately remove the tooth pick and dispose in plastic bag. After adding the sample, add 20.0 µL of mineral oil in each tube and close the cap.

In order to prepare control solution,

i) Add 20.0 µL of mineral oil and close the cap before adding sample in negative control test tube.

ii) Add the sample with tooth pick and then 20.0 µL of mineral oil in each tube, and close the cap.

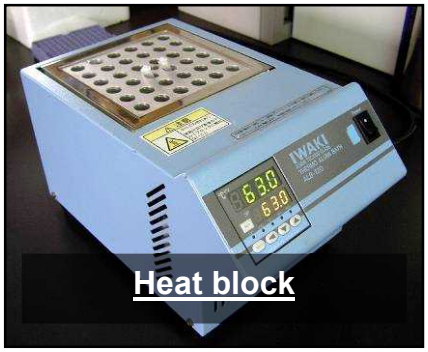
iii) In the end, add 1.0µL of TYLCV positive control and then 20.0 µL of mineral oil in each tube, and close the cap.



Judgement will be difficult if the tooth pick is soaked in the test solution for a while because the tooth pick adsorb the solution and the amount of solution decrease. Do not leave the tooth pick in the test solution after adding sample.

If mineral oil is not added, reaction efficiency will significantly decrease because of condensation of the solution caused by evaporation. Please add mineral oil which is attached in this kit when doing the test.

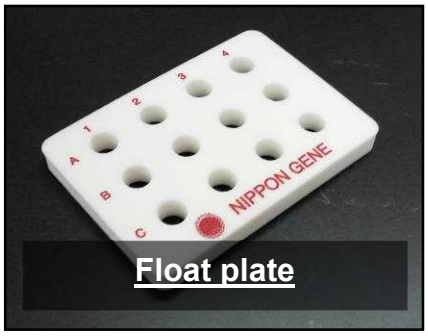
Test Reaction



Heat block

Close Test Tube and mix the reagents by tapping or vibrating on a vortex mixer for about 1 second x 3 times, and spin down the tube. Incubate Test Tube at 63˚C for 60 minutes in the incubator (Air incubator, heating block, or water bath).

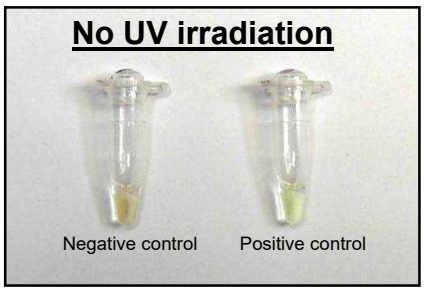
In case of using water bath, use a float plate to prevent the Test Tube tilted on the water surface.

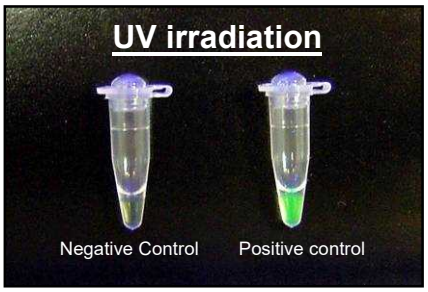


Float plate

Judgement

Judgement of success or failure of the test





After 60 minutes incubation, terminate the reaction by heating for 2 minutes at 80˚C.

Fluorescence Detection Solution before reaction looks light red. If TLCV exists, it turns to vivid green under UV irradiation.

In this procedure, UV transilluminator (wavelength: 240 - 260 nm or 350 - 370 nm), UV goggles, or a face shield is needed.

First, please confirm that Positive Control Solution emits fluorescence and Negative Control Solution does not emit fluorescence. In case which it does not satisfy this condition, the result is invalid. Investigate the cause.

Important

To avoid false result, judge the result immediately after the reaction is completed.

Judgement of the sample

After judgement of the control solutions having done and the test is judged as valid, do the judgement of the results of sample. If Test Solution emits the fluorescence that is same level with the Positive Control Solution under UV irradiation, the sample may contain TYLCV. If the fluorescence is NOT detected under UV irradiation, the sample does not contain TYLCV.

[Tips of judgement]

Test Solution emits fluorescence clearly

The plant may be infected with TYLCV. Even if the emission is weak, when difference can be observed between negative control solution and sample solution, there might be infection.

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

The plant may not be infected with TYLCV.

However, there is a case fluorescent coloring is not recognized because of poor concentration of virus at the beginning of infection. Retest the sample 10 days after in case the sample is suspected to be infected even if being judged “TYLCV negative” by this kit and there is no symptom.

5. Troubleshooting

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

Problem	Possible cause and solution
Test solution gives green color and cannot judge the result	**A. Too much sample is added in test solution** Reaction of this kit is so sensitive that judgement can be done with the tooth pick whose edge is not green. Too much carrier of sample may cause false positive.
Control test solution does not give the right coloring.	**A. Too much TYLCV Positive Control added to the test solution** There are some cases that efficiency of test reaction decreases when too much TYLCV 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, and cleaning procedure by 1% sodium hypochloride aqueous solution are recommended to remove completely the contaminants. After the removal, redo the test. **C. Chelete compounds or metal ion in the sample** The Fluorescent Detection 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 fluorescent detection solution	**A. Judgement not immediately after test reaction has ended** Fluorescent Detection Solution irregularly gains or loses its coloring when left in room temperature for long time. Follow the instruction for storage and handling of the solution.
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).

6. Reference

1. Fukuta S, Kato S, Yoshida K, Mizukami Y, Ishida A, Ueda J, Kanbe M, Ishimoto Y. (2003) Detection of tomato yellow leaf curl virus by loop-mediated isothermal amplification reaction. *J Virol Methods*. **112** (1-2), 35

2. 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

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

7. Supplementary data

[Quality control]

It has been confirmed that the emission of fluorescence was detected when DNA amplification reaction was done with attached Fluorescent Detection Solution, and 1.0 µl of TYLCV Positive Control as template in 25.0 µl reaction volume under 63˚C temperature for 60 minutes.

[Phyletic line]

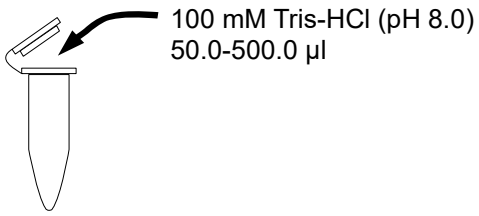
LAMP method needs 4 to 6 primers. The primers contained in this kit is designed based on well preserved region in the strains below included in TYLCV genomic DNA.

GenBank Accession No.	Strain Name
AB014347	TYLCV-Aichi (Aichi Prefecture)
AB116633	TYLCV-Atu (Aichi Prefecture)
AB014346	TYLCV-Shizuoka (Shizuoka Prefecture)
AB110218	TYLCV-Israel Sz (Shizuoka Prefecture)
AB116632	TYLCV-SzY (Shizuoka Prefecture)
AB116635	TYLCV-SzD (Shizuoka Prefecture)
AB116636	TYLCV-SzOs (Shizuoka Prefecture)
AB116634	TYLCV-Kis (Mie Prefecture)
AB110217	TYLCV-Israel Ng (Nagasaki Prefecture)
AB116630	TYLCV-Omu (Nagasaki Prefecture)
AB116629	TYLCV-Miy (Miyazaki Prefecture)
AB116631	TYLCV-Mis (Kumamoto Prefecture)
AB192965	TYLCV-Tos (Kochi Prefecture)
AB192966	TYLCV-Tos (H) (Kochi Prefecture)
AY530931	TYLCV-Florida
NC_004005	TYLCV-Alm
AJ489258	TYLCV-Almeria
AJ223505	TYLCV-Cuba
AF024715	TYLCV-Dominican
AY594174	TYLCV-Egypt
AJ132711	TYLCV-Iran
AF105975	TYLCV-Portugal
AY134494	TYLCV-Puerto Rico
AJ865337	TYLCV-Reunion
AF071228	TYLCV-Spain7297
X61153	TYLCV-Sardinia V

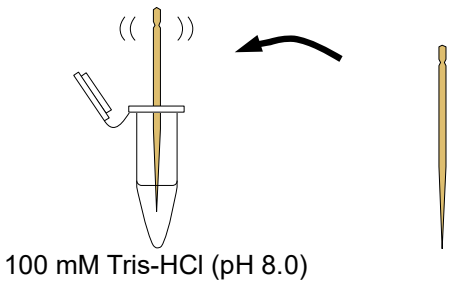
【Other methods for sample preparation】

Tooth pick suspension method

Add 100 mM Tris-HCl (pH 8.0)

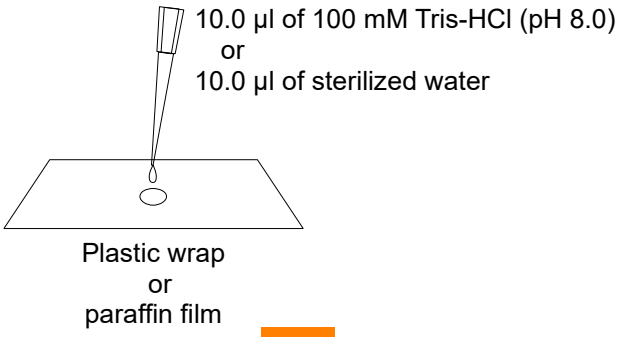


Suspend the tooth pick which pricked tomato leaf

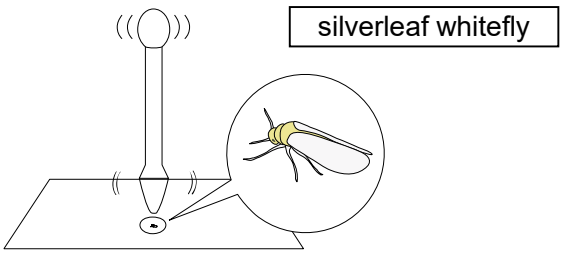


Vector grinding method

Drop 100 mM Tris-HCl (pH 8.0) or sterilized water

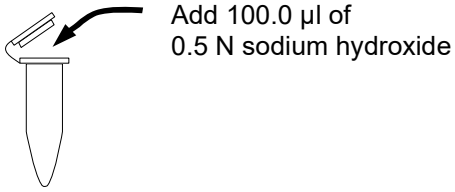


Put silverleaf whitefly and grind with pestle

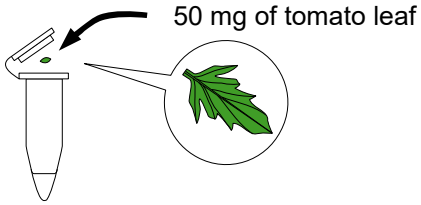


Alkaline grinding method

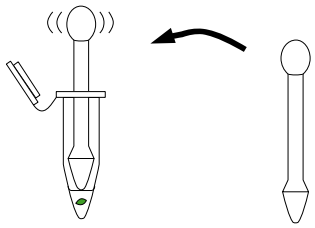
Add 0.5 N sodium hydroxide



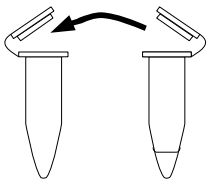
Add tomato leaf



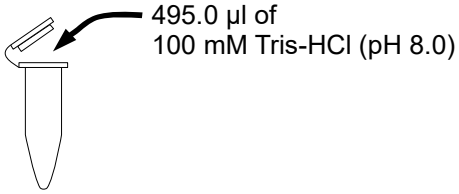
Grind with pestle



Transfer grinded solution to new tube

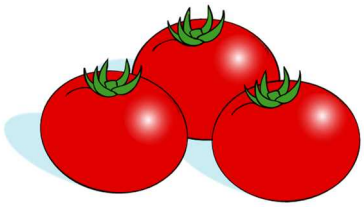


Neutralize with 100 mM Tris-HCl (pH 8.0)



[Memo]

NIPPON GENE



- It may change without a preliminary announcement about the written contents of the description, product specification, and a price.
- “Nippon Gene” is the registered trademarks of Nippon Gene Co., Ltd. in Japan.
- Other proper nouns are the registered trademarks or trademarks of each company.

Information
NIPPON GENE CO., LTD. Address: 2-7-18, Toiya-machi, Toyama 930-0834 Japan TEL: +81-76-451-6548 FAX: +81-76-451-6547 URL: https://nippongene-analysis.com/en/ E-mail: support@nippongene-analysis.com