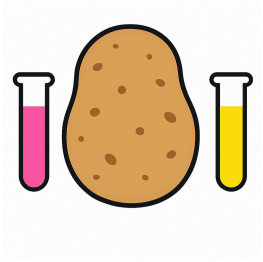
Nov 14, 2025

RT-LAMP for Potato Virus Y detection



DOI
https://dx.doi.org/10.17504/protocols.io.4r3l21je4g1y/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.4r3l21je4g1y/v1

Protocol Citation: Md Munir Mostafiz, Louise McNamara, Stephen Byrne 2025. RT-LAMP for Potato Virus Y detection. **protocols.io** https://dx.doi.org/10.17504/protocols.io.4r3l21je4g1y/v1

Protocol status: Working
We use this protocol and it's working

Created: November 14, 2025

Last Modified: November 14, 2025

Protocol Integer ID: 232419

Keywords: lamp assay, aphid, crop sample, lamp

Funders Acknowledgements:
Marie Skłodowska-Curie Actions (MSCA)
Grant ID: 101106698

Disclaimer

Abstract

A rapid test was developed primarily to support rapid identification of Potato Virus Y in aphid or crop samples. A colorimetric RT-LAMP assay was developed to detect the presence of Potato Virus Y (PVY).

Primers

1 The primers for RT-LAMP were designed for PVY from the sequence MT264731.1 (Potato virus Y isolate P026).

	Primer Name	Sequence (5' to 3')	Scale	Desalting
	F3-PVY001	ACAGTTTGAGCGTTCTGC	25nm	STD
	B3-PVY001	GAATGTGCTGCCTGGTAT	25nm	STD
	FIP-PVY001	CAATTTCTGTGGAAAAACAGGGAAAATTAAAGGTAGGGACATCATCC	25nm	STD
	BIP-PVY001	GAACGAAAGAGTTTGTTTGGTTGGGTAGTGCTTGTTTCTGTGAT	25nm	STD
	LB-PVY001	GACCAACTTTCAGGAGAAGTATGCA	25nm	STD

Primers as ordered from Integrated DNA Technologies (IDT) for Potato virus Y (PVY) assay.

2 A 10X LAMP primer mix was prepared for the assay according to the following table:

	Primer Name	10X Stock (µM)	1X Reaction (µM)
	F3-PVY001	2	0.2
	B3-PVY001	2	0.2
	FIP-PVY001	16	1.6
	BIP-PVY001	16	1.6
	LB-PVY001	4	0.4

Preparation of primer mix for Potato virus Y (PVY) assay. This is labelled as PVY001 10X mix.

Gene Fragments

3 A gene fragment was synthesised for the target region to act as a positive control.

LAMP-PVY001.fasta 521B

These were ordered from Integrated DNA Technologies and diluted to 10 ng/µL with IDTE (Concentration 10 millimolar (mM) Tris, Concentration 0.1 µg/µL EDTA). Working solutions for testing were prepared by carrying out serial dilutions of the stock.

RT-LAMP Reaction

4 Thaw all components at RT and place on ice (WarmStart Colorimetric LAMP 2X Master Mix; 10X LAMP primer mix; Target RNA or gBlock (0.1 ng/µL) controls.

5 Prepare reaction mix according to table below for the number of target samples (plus positive control(s) and no template and/or negative control(s)):

	Component	Amount (µl)
	WarmStart Colorimetric LAMP 2X Master Mix	12.5
	LAMP Primer Mix (10X) [MAV0001 or PAS0001]	2.5
	dH2O	9

Vortex reaction mix and briefly spin contents down in centrifuge.

6 Pipette 24 µL into PCR 8-strips (e.g. 72.991.002 from Sarstedt) and add 1 µL of target RNA, gBlock, control, or water for no-template-control. Mix by pipetting up and down, and briefly centrifuge to collect contents. Confirm that reaction solutions have a bright pink color, which indicates an initial high pH, which is required for a successful pH-LAMP reaction.

7 Seal reaction vessels.

8 Incubate at 65 °C in a thermocycler or heating block for 30 minutes.

9 Remove tubes or vessels from incubation and examine by eye. Positive reactions will have turned yellow while negative controls should remain pink. If color change is not robust, e.g. an orange color is visible, return reactions to 65 °C for an additional 00:10:00 . When extending the reaction time it is important to monitor the no-template and negative controls closely to ensure false positive reactions are not present. Color will be visible directly on removal from incubation temperature, but can be intensified by allowing the reaction to cool to room temperature.

10 The result can be photographed to record the colorimetric results, or simply kept at room temperature in the reaction vessel.

Image of RT-LAMP result - two positive tubes on left (yellow) and two negative tubes on right (pink).