PhytoDETECT® TSWV RT-qPCR Kit

(Tomato Spotted Wilt Detection by RT-qPCR)

TBK1076. 100 reactions

Introduction

Tomato Spotted Wilt Virus (TSWV) is one of the most devastating viral pathogens. It reduces the productivity and quality of important ornamental crops and vegetables, being capable of infecting over 550 botanical species. The infection is characterized by the formation of chlorotic rings on the leaves that necrose with a bronzed color, and the fruits lose their commercial value. It is transmitted by at least nine species of thrips and is widely distributed around the world. TSWV belongs to the genus Tospovirus and has a genome composed of 3 RNA segments known as Large (L), Medium (M), and Small (S).

The PhytoDETECT® TSWV RT-qPCR Kit enables the detection of TSWV through a real-time quantitative RT-PCR reaction. The kit includes a master mix containing the necessary enzymes, optimized primers and probes, as well as a DNA-based positive amplification control (PAC) to ensure that the PCR amplification is performed efficiently with the supplied components.

Features

- One-tube cDNA synthesis and PCR reaction.
- Compatible with all real-time thermocyclers.
- TSWV detection in FAM channel.
- 100% inclusivity: *Tospoviruses* serogroups I y II
- 100% exclusivity: Tospoviruses of more distant serogroups (III, IV, VI, and VIII)

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Kit Components

Description	TBK1076
qPCR Probe Master Mix (2x)	1 mL
ROX Reference	1 vial
RT Mix	100 µL
TSWV Primers & Probe Mix (10x)	200 µL
TSWV_PAC (Control Positivo)	1 vial
Water, nuclease free	1 mL

Order Info Kit Components: qPCR Probe Master Mix (TBZ0350) | ROX Reference (TBR0278) | RT-Mix (TBZ0352) | TSWV Primers-& Probe Mix (10x) (TBK1076-1) | TSWV_PAC (TBK1076-2) | Water, nuclease free (TBB0302).

Storage

PhytoDETECT® TSWV RT-qPCR Kit is shipped with cold gel packs. Upon receipt, store the kit at -20°C. Avoid repeated freeze-thaw cycles. The TSWV Primers & Probe Mix is light-sensitive and should be stored in the dark.

Virus		Serogroups	Serotypes
TSWV	Tomato Spotted Wilt Virus (BR-01, CNPH, TSWV-L3)	I	
TCSV	Tomato chlorotic spot virus (BR-03)	II	Tipo I
GRSV	Groundnut ringspot virus (TSWV-groundnut, SA-05)	II	Tipo II
INSV	*Impatiens necrotic spot virus* (TSW-I, NL07)	III	
WSMoV	*Watermelon silver mottle virus*	IV	
IYSV	*Iris yellow spot virus*	VI	
CSNV	Chrysanthemum stem necrosis virus	VIII	

Required Materials (Not Included)

- Filter tips
- Optical-grade PCR tubes/ plates

PROTOCOL

Technical Recommendations

- *RNA extraction is **mandatory** before using the **PhytoDETECT® TSWV RT-qPCR Kit**.*
- *The quality of the extracted RNA significantly impacts the overall assay performance. Ensure that the nucleic acid extraction system used is compatible with RT-qPCR.*
- *Include an **internal extraction control** when performing RNA extraction.*

A. RT-qPCR

1. Thaw all kit components on ice. Mix each solution thoroughly and briefly spin down the tubes.
2. Use the following reaction setup for a 20 µL reaction volume:

Component	Reaction Volume*
qPCR Probe Master Mix (2x)	10 µL
RT Mix	1 µL
TSWV Primers & Probe Mix (10x)	2 µL
Water, nuclease free	Up to 15 - 18 µL

** Prepare a mix for all reactions, considering two additional reactions for controls. Use ROX if required by the thermocycler.*

3. Distribute **15-18 µL** of the prepared mix into the required number of tubes/wells. Include one well for NAC and one for PAC (*see notes*).

   *Use **5 µL of a TSWV_PAC dilution (1:10)** (Positive Amplification Control).*
4. Add **2-5 µL** of extracted RNA sample to each reaction tube and mix well.

   *The quality of the test depends on the quality of the RNA sample. Improper collection, storage, or transport of samples can lead to **false negatives**.*
5. Place the tubes in the thermocycler and set up the following real-time PCR program:

Step	Temperature	Time	Cycles	Detection
Reverse Transcription	50 °C	30 min	1x	
Initial Activation	95 °C	5 min	1x	
Denaturation	95 °C	15 sec	40x	
Annealing & Extension	60 °C	1 min		FAM

B. Amplification Monitoring & Data Analysis

1. To monitor amplification in real-time, fluorescence should be measured in the FAM channel (Excitation 495 nm / Emission 520 nm), following the thermocycler's user manual. Results should be interpreted as follows:

	TSWV Presence	TSWV Absence
PAC (Positive Control)	+	+
NAC (Negative Control)	C_T= N/A	C_T= N/A
Sample	$C_T \leq 35$	$C_T > 35$

Notes:

- Positive Amplification Control (PAC): Ensures PCR efficiency. The PhytoDETECT® TSWV RT-qPCR Kit includes a DNA-based TSWV_PAC.
- Negative Amplification Control (NAC): Prevents false positives due to contamination. Use nuclease-free molecular biology water.