

PhytoDetect™ CGMMV RT qPCR Kit

Cucumber Green Mottle Mosaic Virus Detection by RT-qPCR

TBK1084, 100 reactions

Introduction

PhytoDETECT™ CGMMV RT-qPCR Kit enables the detection of cucumber green mottle mosaic virus (CGMMV) 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.

Cucumber Green Mottle Mosaic Virus is a pathogen belonging to the family *Virgaviridae* that affects a wide range of cucurbit crops, especially cucumbers, melons, and squash. It is characterized by causing green mosaic spots on the leaves, fruit deformation, and overall stunted growth of the plant, resulting in significant losses in yield and quality in the affected crops. CGMMV is highly contagious and is easily transmitted through direct contact between plants, contaminated agricultural tools, and infected seeds. Early detection with effective diagnostic tools is essential to manage the spread of this virus and minimize its economic impact on agriculture.

Features

- One-tube cDNA synthesis and PCR reaction.
- Compatible with all real-time thermocyclers.
- CGMMV detection in **FAM** channel.

Kit Components

Description	TBK1084
qPCR Probe Master Mix (2x)	1 mL
ROX Reference	1 vial
RT Mix	100 µL
CGMMV Primers & Probe Mix (10x)	200 µL
CGMMV_PAC (Positive Control)	1 vial
Water, nuclease free	1 mL

Order Info Kit Components: qPCR Probe Master Mix (TBZ0350) | ROX Reference (TBR0278) | RT-Mix (TBZ0352) | CGMMV Primers-& Probe Mix (10x) (TBK1084-1) | CGMMV_PAC (TBK1084-2) | Water, nuclease free (TBB0302).

Storage

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

Required Materials (not included)

- Filter tips
- Optical-grade PCR tubes/ plates

Technical Support: info@tiarisbiosciences.com

PROTOCOL

Technical Recommendations

- RNA extraction is **mandatory** before using the **PhytoDETECT™ CGMMV 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
CGMMV 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 CGMMV_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	20 min	1x	
Initial Activation	95 °C	5 min	1x	
Denaturation	95 °C	5 sec	35x	
Annealing & Extension	58 °C	25 sec		

B. Amplification Monitoring & Data Analysis

- 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:

	CGMMV Presence	CGMMV Absence
PAC (Positive Control)	+	+
NAC (Negative Control)	$C_T = N/A$	$C_T = N/A$
Sample	$C_T \leq 33$	$C_T > 33$

Notes:

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