# PhytoDETECT™ ToBRFV RT-qPCR Kit

## Tomato Brown Rugose Fruit Virus Detection Kit by RT-qPCR

TBK1070-50. 50 reactions

TBK1070-100. 100 reactions

### Introduction

PhytoDETECT™ ToBRFV RT-qPCR Kit enables the detection of ToBRFV 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.

The Tomato Brown Rugose Fruit Virus (ToBRFV) is an emerging and rapidly spreading RNA virus that infects tomato and pepper plants, with tomato being the primary host. This virus causes severe crop losses and threatens tomato production worldwide. ToBRFV was first identified in greenhouse-grown tomato plants in Jordan in the spring of 2015, with the earliest outbreak traced back to Israel in 2014. To date, the virus has been reported in at least 35 countries across four continents.

### Features

- One-tube cDNA synthesis and PCR reaction
- Compatible with all real-time thermocyclers
- ToBRFV detection in FAM channel
- 100% inclusivity
- 100% exclusivity against other Tobamoviruses, including Tomato Mosaic Virus (ToMV), Pepper Mild Mottle Virus (PMMoV), and Cucumber Green Mottle Mosaic Virus (CGMMV). Other host-range viruses such as TSWV, PepMV, TYLCV, TBSV, ToRSV, and TNV are also excluded.

### Kit Components

| Components | 50 rxn | 100 rxn |
|---|---|---|
| qPCR Probe Master Mix (2x) | 0.5 mL | 1 mL |
| ROX Reference | 1 vial | 1 vial |
| RT Mix | 50 µL | 100 µL |
| ToBRFV Primers & Probe Mix (10x) | 100 µL | 200 µL |
| ToBRFV_PAC (Positive Control) | 1 vial | 1 vial |
| Water, nuclease free | 1 mL | 1 mL |

***Order Info Kit Components:*** *qPCR Probe Master Mix (TBZ0350) | ROX Reference (TBR0278) | RT-Mix (TBZ0352) | ToBRFV Primers-& Probe Mix (10x) (TBK1070-1) | ToBRFV_PAC (TBK1070-2) | Water, nuclease free (TBB0302).*

### Storage

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

### Required Materials (*not included*)

- Filter tips
- Optical-grade PCR tubes/ plates
- Optical sealing film

**Technical Support:** info@tiarisbiosciences.com

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## PROTOCOL

### Technical Recommendations

- *RNA extraction is **mandatory** before using the **PhytoDetect™ ToBRFV 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:

| Components | Reaction Volume* |
|---|---|
| qPCR Probe Master Mix (2x) | 10 µL |
| RT Mix | 1 µL |
| ToBRFV 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 it is 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 ToBRFV_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 | 60 °C | 25 sec | | 📷 |

## 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:

| | **ToBRFV Presence** | **ToBRFV 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. **PhytoDetect™ ToBRFV RT-qPCR Kit** includes a DNA-based ToBRFV_PAC.
- Negative Amplification Control (NAC): Prevents false positives due to contamination. Use nuclease-free molecular biology water.