

ASSAY NAME: PMMoV (Control assay)

Quantity: 100 x 20µL PCR reactions

Singleplex assay: PMMoV

SKU#’s:

BUN-PMMV-P

(RUO). Research Use Only. Not for use in Diagnostic Procedures.

SCOPE OF THIS DOCUMENT

The oligonucleotide recipes are optimized for each instrument (BioRad, QuantStudio, MIC). The verification data presented in this document were performed using SKU: PNP-PMMV-P on a BioRad CFX96. The performance of the other SKUs on their corresponding instrument should be similar. Contact PCRassays.com if you need to use a different qPCR instrument.

INTRODUCTION

The pepper mild mottle virus (PMMoV) is a plant (+)sense RNA virus that is highly abundant in human feces. It serves as a reliable internal control when detecting RNA pathogens (e.g. RNA viruses or other RNA) by RT-qPCR (reverse transcription quantitative polymerase chain reaction).^{1, 2, 3}

For detecting DNA viruses, bacteria and fungi in wastewater, we recommend our CrAssphage control assay (SKU: PNP-CRPH-BR).

CONTENTS

The primers and probes in the PMMV assay are provided in Tube 1 as a 20X concentrated working solution that detects PMMoV

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
PMMoV	HEX	BHQ-1	4, 5

The probes are designed as TaqMan⁶ cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity.

KIT HANDLING AND CONTAMINATION

The PMMV bundle is shipped on ice, and should be stored at -20 °C. The tubes should be kept on ice once thawed. Do not subject the enzyme to multiple freeze-thaw cycles.

Any contamination should be avoided by using appropriate personal protective equipment (PPE), powder free gloves, aerosol barrier pipette tips, and a clean hood.

Assay contents:

Tube 1: 20X Primer/Probe mix specific for PMMoV genomic RNA.

Tube 2: (Do NOT add to specimen unknowns) Positive control: Synthetic 500 bp DNA fragment of PMMoV

Tube 3: InhibiTaQ Standard RT-qPCR enzyme Mastermix (enough for 100 rxns. with 20 µL total volume). This is a custom formulation from Fortis Life Sciences to the specifications of PCRassays.com.



EXPERIMENTAL

Perform nucleic acid extraction/purification (recommended).

Set up your reaction (20 µL) as follows on ice:

Component	Volume (µL)
InhibiTaQ RT-qPCR enzyme mastermix (2X)	10
Primer/Probe mix (20X)	1
Sample	2
Water (Molecular biology grade)	7

Notes: To improve assay sensitivity, up to 9 µL of sample can be added (water volume adjusted accordingly) for a total reaction volume of 20 µL. For positive control rxns., add 2 µL of the solution from Tube 2. For 10 µL reactions, divide all of the amounts above by a factor of 2.

A RT-qPCR protocol was used for verification on a Bio-Rad CFX96™ Real-Time System, with the following program:

Step	Thermocycling Protocol:
1	Incubate @ 50 °C for 5 minutes
2	Incubate @ 94 °C for 3 minutes
3	Incubate @ 94 °C for 5 seconds
4	Incubate @ 55-63 °C for 15 seconds
5	Plate Read
6	Go to Step 3, repeat 44x more
7	(optional) Incubate @63 °C for 3 minutes

For QuantStudio instruments, we recommend a Step 3 cycle time of 22 seconds at 55-63 °C.

RESULT INTERPRETATION

After running the qPCR reaction, perform a regression analysis on the data to determine the quantification cycle, C_q. (C_q is preferred over C_t). Each fluorescence channel with a C_q < 38 cycles and final RFU > “threshold” is considered “positive” or “+” in the Table below. The “threshold” is 2.0 on the BMS MIC, 200 on BioRad instruments and 200,000 on QuantStudio 5, 6, 7, 12K instruments.

PMMoV HEX TM	Recommended Interpretation
—	The PCR reaction failed. Please repeat the experiment
+	The sample contains PMMoV RNA

VERIFICATION EXPERIMENTS

Experiments were performed in triplicate using the thermocycling protocol given above. Only primers and probe from the PMMoV RNA assay were employed, without any primers or probe for other targets. The samples used for the validation experiments included water (NTC) and three 10X diluted RNA extract solution from 3 local wastewater samples (generously provided by Life Magnetics, Inc.). The results of these experiments are shown in **Figure 1**. The PMMoV assay described herein has been tested with a variety of pathogen targets (e.g. see PCR assays.com products for SARS CoV2).

Conclusion: The data in **Figure 1 and 2** indicate that the PMMoV assay can detect PMMoV genomic DNA in wastewater samples and serve as an internal control assay in multiplexed PCR reactions.

NOTES

¹D'Aoust PM, Mercier E, Montpetit D, *et al.* Quantitative analysis of SARS-CoV-2 RNA from wastewater solids in communities with low COVID-19 incidence and prevalence. **Water Res.** (2021) 188, 116560.

²Rosario, K., Symonds, E.M., Sinigalliano, C., *et al.* Pepper Mild Mottle Virus as an Indicator of Fecal Pollution. **Appl. Environ. Microbio.** (2009) 75 (22), 7261.

³Ahmed W., Bivins, A., Bertsch, P.M., *et al.* Surveillance of SARS-CoV-2 RNA in wastewater: Methods optimization and quality control are crucial for generating reliable public health information. **Curr. Opin. Environ. Sci, Health.** (2020) 17, 100209.

⁴HEXTM (Hexachloro-fluorescein) is a trademark of Applera Corp.

⁵BHQ-1TM (Black Hole Quencher) is a trademark of Biosearch Technologies, Inc.

⁶“TaqMan” is a trademark of Roche Molecular Systems, Inc.

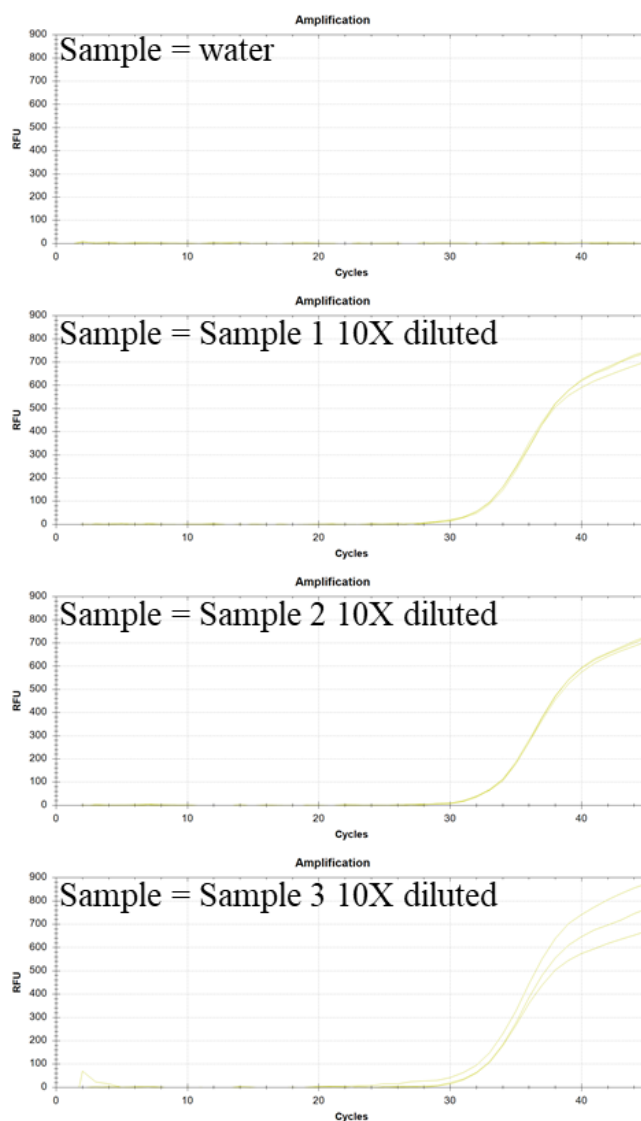


Figure 1: Verification experiments with water (no reaction observed) and three RNA extract samples from wastewater from three different locations in Michigan (for PCR each sample was 10X diluted). The HEX probe detects PMMoV genomic RNA.

CONTACT US

For assistance, please contact DNA Software using the link:
<https://dnasoft.jira.com/servicedesk/customer/portals>

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