SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

PCRassays.com

ASSAY NAME: PMMoV (Control assay)

Quantity: 100 x 20µL PCR reactions

1-plex assay: A positive control designed for wastewater samples to specifically detect PMMoV genomic RNA in singleplex or multiplex reactions.

SKU#'s:
PNP-PMMOV-BR (Bio-Rad CFX instrument)
PNP-PMMOV-QS (QuantStudio instrument)
PNP-PMMOV-MIC (BMS MIC instrument)

(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-PMMOV-BR 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

Tube 1 contains a mixture of primers/probe targeting PMMoV genomic RNA is provided in a tube (a 20X concentrated working solution). The probes are designed as TaqMan[6] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity (we recommend InhibiTaq Standard RT-qPCR Master Mix).

Table of Dyes used in this assay:

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

Tube 2 "positive control" contains a synthetic 500 bp DNA construct containing the amplicon regions of PMMoV genomic RNA (AB000709.2) is provided as a positive extraction control. The concentration of this DNA construct is approximately 5,000 copies/µL. The Control DNA constructs are for validation purposes only and **Tube 2 should NOT be added to wells for specimen unknowns**.

Assay contents:

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

Tube 2: (optional if ordered) 5000 copies/µl Positive control of synthetic 500 bp DNA fragment of PMMoV (NCBI accession: AB000709.2).

Tube 3: (optional if ordered) 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.

ASSAY HANDLING AND CONTAMINATION

The PMMoV assay is shipped at ambient temperature, and should be stored at -20 °C. The assay 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.

Note: molecular biology grade water should be used to prepare the PCR reactions (NOT included in this assay).

EXPERIMENTAL

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

Component	Volume (µL)
InhibiTaq Standard RT-qPCR enzyme mastermix (2X)	10
PMMoV Primer/Probe mix (20X)	1
(optional) Pathogen Primer/Probe mix (20X)	1
Sample	2
Water	6

Notes: To improve assay sensitivity, up to 8 µL of sample can be added (adjust water volume) for a total reaction volume of 20 µL. For positive control rxns., add 2 µL of the solution from Tube 2 (i.e., the "sample").

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, Cq. (Cq is preferred over Ct). Each fluorescence channel with a Cq < 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.

Sample results with a hypothetical Pathogen and using PMMoV as the internal control.

Target Pathogen Fluorphore™	hRPP30 HEX™	Recommended Interpretation
–	–	The PCR reaction failed. Please repeat the experiment
–	+	The sample doesn't contain the target RNA.
+	–	The sample contains the Pathogen RNA. The sample may not contain PMMoV RNA.
+	+	The sample contains the Pathogen RNA, and 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.

==========

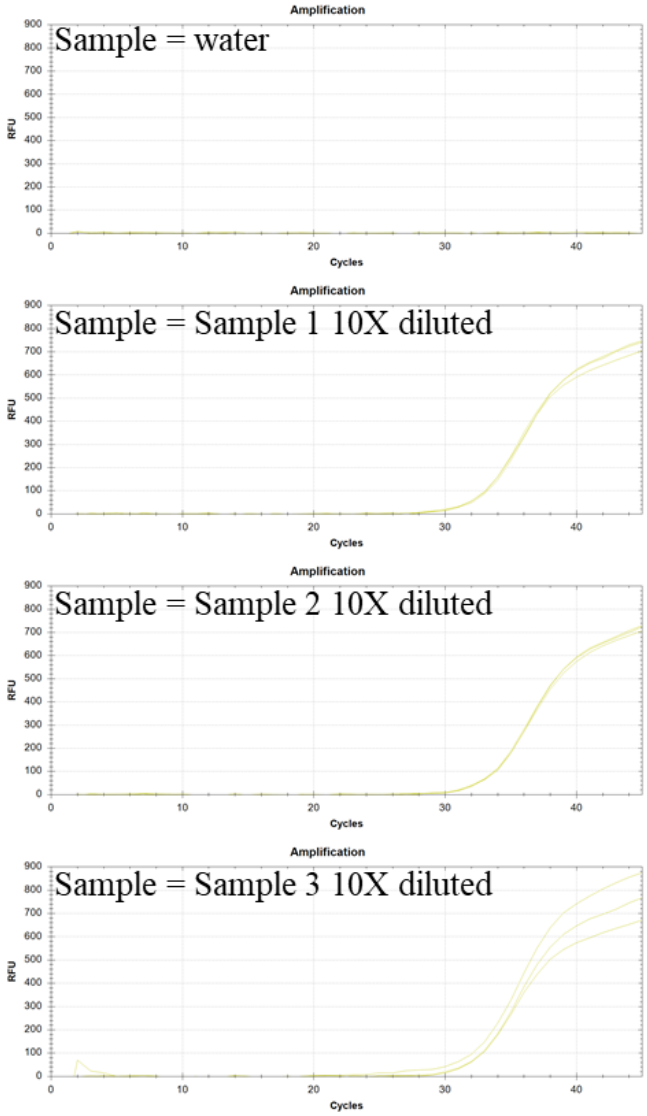

Figure 1: Verification experiments with water (no reaction observed) and three RNA extract samples from wastewater from three different locations in Michigan (for PCR each sample was 10X diluted). The HEX probe detects PMMoV genomic RNA.

==========

NOTES

1. D'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.
2. Rosario, 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.
3. Ahmed 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.

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

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

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

CONTACT US

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

Address: Michigan Life Science and Innovation Center,
46701 Commerce Center Dr, Plymouth, MI 48170

Phone: (734) 222-9080