ASSAY NAME: CrAssphage

Quantity: 100 x 20µL PCR reactions

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

SKU#'s:
PNP-CRPH-BR (Bio-Rad CFX instrument)
PNP-CRPH-QS (QuantStudio instrument)
PNP-CRPH-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-CRPH-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 CrAssphage are a family of double-stranded DNA viruses that is highly abundant in human feces and wastewater. It serves as a reliable internal control when detecting DNA from various DNA pathogens (e.g. bacteria, fungi, and DNA viruses) by qPCR reaction.

For detecting RNA viruses in wastewater, we recommend our control assay for PMMoV (SKU: PNP-PMMOV-BR).

CONTENTS

A mix of primers/probe targeting CrAssphage genomic DNA (the gene of a hypothetical protein, ID: YP_009052522.3) is provided in a tube (a 20X concentrated working solution). The fluorophore of the probe is **HEX**™ (Hexachloro-fluorescein, a trademark of Applera Corp.), and the quencher is BHQ-1™ (Black Hole Quencher, a trademark of Biosearch Technologies, Inc.). The probes are designed as TaqMan[3] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity (we recommend InhibiTaq Standard qPCR Master Mix).

Assay contents:

Tube 1: 20X Primer/Probe mix specific for CrAssphage genomic DNA.

Tube 2: (optional if ordered) 5000 copies/µl Positive control of synthetic 500 bp DNA fragment of CrAssphage (NC_02471).

Tube 3: (optional if ordered) InhibiTaq Standard 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.

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
CrAssphage control	**HEX**	BHQ-1	1, 2

Tube 2 "positive control" contains a synthetic 500 bp DNA construct (from Twist Biosciences) containing the amplicon regions of CrAssphage genomic DNA (NC_02471) 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 HANDLING AND CONTAMINATION

The CrAssphage 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 qPCR enzyme mastermix (2X)	10
CrAssphage 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 (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 (i.e., the "sample").

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

Step	Thermocycling Protocol:
1	Incubate @ 95 °C for 2 minutes
2	Incubate @ 95 °C for 3 seconds
3	Incubate @ 55 °C for 15 seconds
4	Plate Read
5	Go to Step 3, repeat 44xmore

For QuantStudio instruments, we recommend a Step 3 cycle time of 22 seconds at 55 °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 CrAssphage as the internal control.

Target Pathogen **Fluorphore**^{M}	hRPP30 **HEX**™	Recommended Interpretation
–	–	The PCR reaction failed. Please repeat the experiment
–	+	The sample doesn't contain the target DNA.
+	–	The sample contains the **Pathogen** DNA. The sample may not contain CrAssphage DNA.
+	+	The sample contains the **Pathogen** DNA, and CrAssphage DNA.

VERIFICATION EXPERIMENTS

Experiments were performed in triplicate using a protocol modified from the experimental procedure given above. The CrAssphage assay was tested as (1) singleplex using 500 BP synthetic DNA construct (from Twist Biosciences) from the CrAssphage genome containing the amplicon, (2) duplex with MPX1 assay (PCRassays.com product for detecting monkeypox virus) using synthetic DNA constructs containing the amplicons from both the CrAssphage and monkeypox genomes (**Figure 1**), and (3) triplex with MPX1 and OPV1 assays (PCRassays.com product for detecting orthopox viruses). The CrAssphage assay was also tested using 7 wastewater extract samples kindly provided by Dr. Jeffrey L. Ram's lab from Wayne State University (**Figure 2**, next page).

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

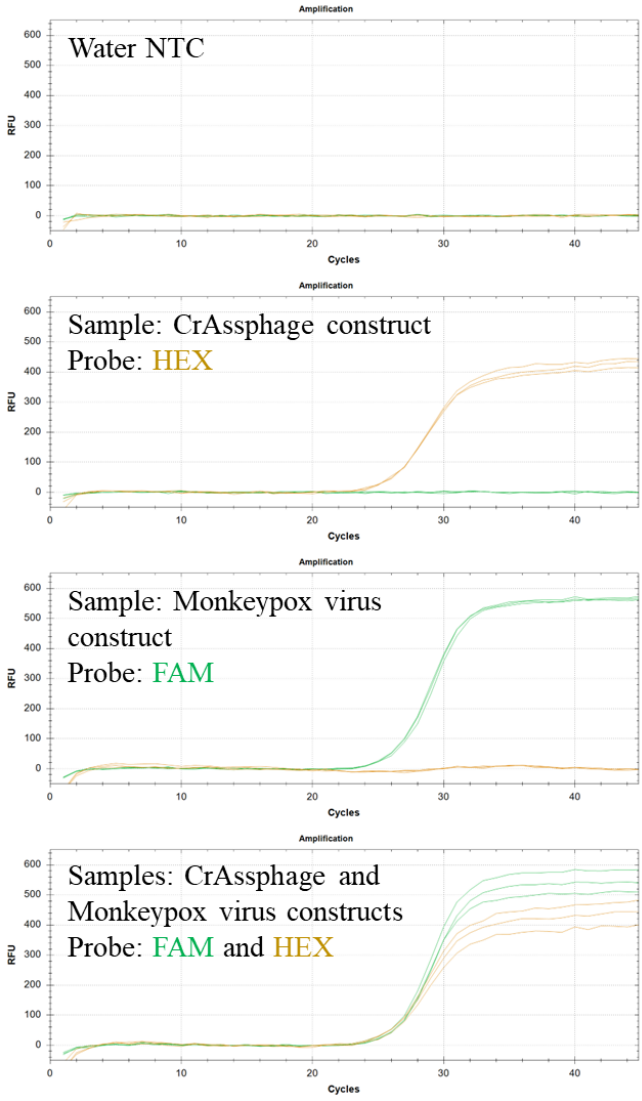

Figure 1: Verification experiments (in a 2-plex reaction of CrAssphage and MPX1 products from PCRassays.com) with various added samples (given in text boxes for each panel). Top panel: No template control (NTC) with water – no PCR reaction observed. Second from top panel: CrAssphage DNA construct where only **HEX** signal was observed (no **FAM** signal). Third from top panel: Monkeypox DNA construct where only **FAM** signal was observed (no **HEX** signal). Bottom panel: both DNA constructs for CrAssphage and Monkeypox were added and both **FAM** and **HEX** signals were observed.

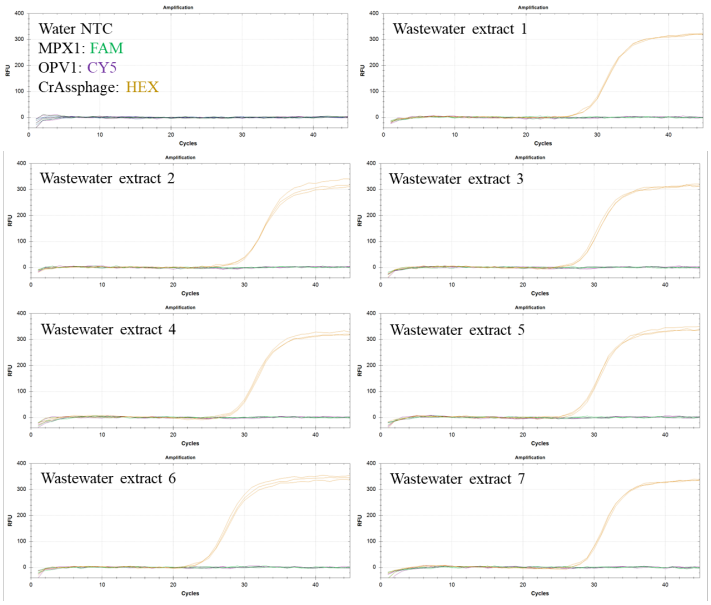

Figure 2: Validation experiments (in a triplex of CrAssphage, MPX1, and OPV1 products from PCRassays.com) with water (no reaction observed) and 7 wastewater extract samples. All wastewater extract samples only showed positive results for CrAssphage.

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

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NOTES

[1] HEX™ (Hexachloro-fluorescein) is a trademark of Applera Corp.

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

[3] "TaqMan" is a trademark of Roche Molecular Systems, Inc.