PCRassays.com

ASSAY NAME: VURP9_QS
(Viral Respiratory Panel 9 for QuantStudio)

Quantity: 100 x 20µL PCR reactions

4-plex assay (3-color): Human metapneumovirus subtypes A and B, Human bocavirus type 1, and human RPP30 DNA

SKU #: PNP-VURP9-D-QS-100 (QuantStudio)

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

SCOPE OF THIS PRODUCT INFORMATION SHEET (PIS):

The oligonucleotide recipes are optimized for each instrument (QuantStudio, BioRad). The verification data presented in this PIS were performed using PNP-VURP9-D-QS-100 on a QuantStudio™ 7 Flex Real-Time System. The performance of the other SKUs on their corresponding instrument should be similar. Contact PCRassays.com if you are planning to use a different instrument.

CONTENTS

The primers and probes in the VURP9_QS assay are provided in Tube 1 as a 5X concentrated working solution that detects 3 pathogens, and a human control.

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
HMPV A and B	FAM	BHQ-1	1,2
RPP30-DNA control	TAMRA	BHQ-2	3,4
HBoV1	TEX615	BHQ-2	5

The probes are designed as TaqMan[6] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity.

ASSAY HANDLING

The VURP9_QS 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.

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: 5X Primer/Probe mix for HMPV A and B, HBoV1, and hRPP30DNA.

Tube 2: (Do NOT add to specimen unknowns) Positive control: 5000 copies/µl of synthetic 500 bp DNA fragments for HMPV A and B, HBoV1, and hRPP30DNA.

Tube 3: InhibiTaq RTqPCR 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 PCR reaction (20 µL) as follows on ice:

Component	Volume (µL)
InhibiTaq enzyme mastermix (2X)	10
Primer/Probe mix (5X)	4
Sample	2
Water (Molecular Biology Grade)	4

Notes: To improve assay sensitivity, up to 6 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.

A PCR protocol was used for verification on a QuantStudio™ 7 Flex Real-Time System, with the following program:

Step	Thermocycling Protocol:
1	Incubate @ 50 °C for 10 minutes
2	Incubate @ 85 °C for 1 second
3	Incubate @ 93 °C for 2 minutes
4	Incubate @ 93 °C for 5 seconds
5	Incubate @ 55 °C for 22 seconds
6	Plate Read
7	Incubate @ 85 °C for 1 second
8	Incubate @ 93 °C for 5 seconds
9	Incubate @ 55 °C for 22 seconds
10	Plate Read
11	Go to Step 7, repeat 43× more

Note: The 85 °C steps are present to reduce temperature overshooting that is common on QuantStudio Instruments.

RESULT INTERPRETATION

After running the qPCR reaction, use the instrument software to determine the quantification cycle, Cq (or use Ct if your instrument does not have the capability to compute Cq). Fluorescence channels with a Cq <38 cycles, and a final RFU > threshold is considered "positive" or "+" in the table below. The "threshold" value for calling a PCR positive is dependent on the instrument model, well size, and sample volume; thus the user must determine the threshold that is appropriate for their method. For our QuantStudio™ 7 Flex with 96 well plate with 100µL wells and 20µL reaction volume, the average RFU was approximately 1,500,000 and we used a threshold of 200,000 for calling positives.

HMPV A/B FAM™	hRPP30 TAMRA™	HBoV1 TEX615™	Recommended Interpretation
–	–	–	The PCR reaction failed. Please repeat the experiment
–	+	–	The sample does not contain pathogen RNA. The sample contains human RPP30 DNA.
+	–	–	The sample contains HMPV A and/or B RNA. The sample may not contain human RPP30 DNA.
+	+	–	The sample contains HMPV A and/or B RNA and human RPP30 DNA.
–	–	+	The sample contains HBoV1 DNA. The sample may not contain human RPP30 DNA.
–	+	+	The sample contains HBoV1 DNA and human RPP30 DNA.
+	–	+	The sample contains HMPV A and/or B RNA, and HBoV1 DNA. The sample may not contain human RPP30 DNA.
+	+	+	The sample contains HMPV A and/or B RNA, HBoV1 DNA, and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The VURP9_QS assay verification was carried out as a 4-plex (3-color) assay, which simultaneously detects RNA from Human metapneumovirus A and B, DNA from human bocavirus type 1, and human RPP30 DNA, which serves as a positive extraction-control assay.

Experiments were performed in triplicate using the experimental procedure given above, but with different samples added to each reaction. The samples used for the verification experiments contained $1x10^4$ copies/reaction of synthetic 500 bp DNA constructs (from Twist Biosciences) harboring the regions of interest from the pathogen genomes, human RPP30-DNA gene, and human genomic DNA. RNA samples were provided by Assurance Scientific Laboratory. The results of these experiments are shown in **Figure 1** and indicate that the multiplex assay specifically detects the different pathogens in the human genomic DNA matrix.

The limit of detection (LOD) was estimated by performing serial dilution experiments in triplicate (**Figure 2**). For dilution series only target construct was added. The results show a limit of detection (LOD) <10 copies/reaction.

Conclusion: The data in **Figure 1** indicate that the 3-plex primers and probes specifically detect and differentiate the pathogens and are also compatible with RPP30_DNA positive control primers.

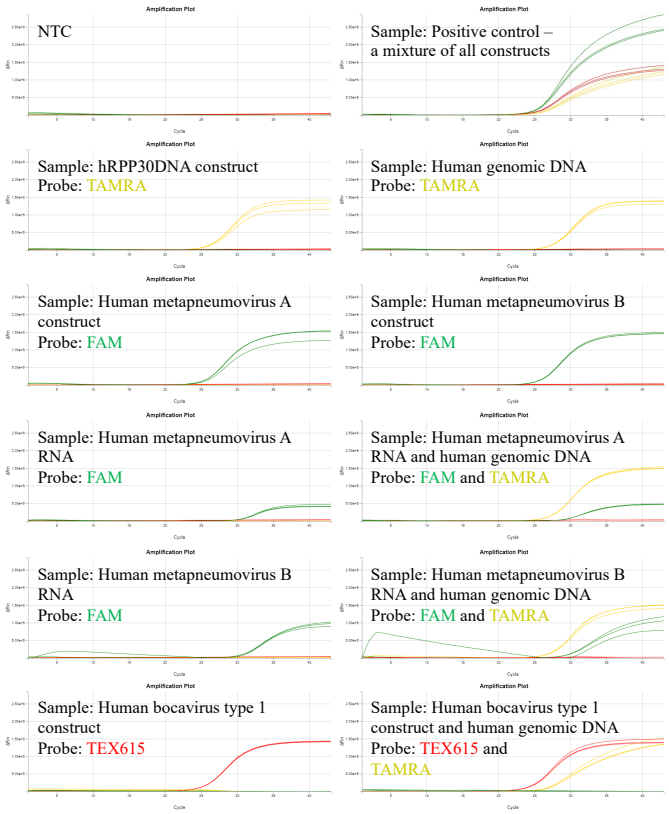

Figure 1: Verification experiments with single targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

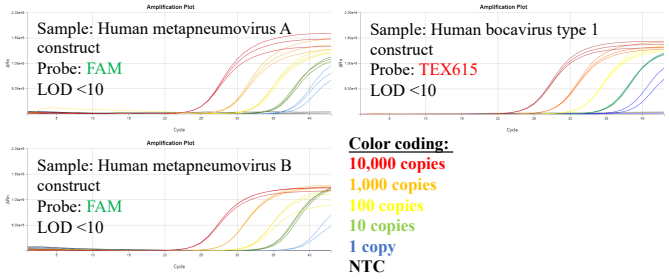

Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

CONTACT US

For assistance, please contact DNA Software using the link: https://www.pcrassays.com/contact/

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NOTES

[1] FAM™ (Carboxyfluorescein) is a trademark of Life Technologies, Inc

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

[3] TAMRA (Carboxyltetramethylrhodamine) is a trademark of Applera Cor.

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

[5] TEX615™ is a trademark of Thermo Fisher Scientific.

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