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ASSAY NAME: RVEV_D_QS (Rhino/Enteroviruses for QuantStudio™)

Quantity: 100 x 20µL PCR reactions

2-color assay Human Rhinovirus types A, B, and C, Enteroviruses A71 and D68 and other A and D types, Coxsackievirus types A and B, Poliovirus serotypes 1, 2, and 3, and human RPP30 DNA

SKU: BUN-RVEV-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 BUN-RVEV-D-QS-100 on a QuantStudio™ 7 Pro Real-Time System. 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.

CONTENTS

The primers and probes in the RVEV assay are provided in Tube 1 as a 5X concentrated working solution that detects human Rhinovirus types A, B, and C, Enteroviruses A71 and D68 and other A and D types, Coxsackievirus types A and B, Poliovirus serotypes 1, 2, and 3. This assay does NOT detect Parechoviruses. All of these pathogens are detected with a single FAM labeled probe but with a mixture of 7 primer sets to amplify all the different types. The assay also detects a human extraction control.

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
Rhino/Enteroviruses	**FAM**	BHQ-1	1,2
RPP30-DNA control	**TAMRA**	BHQ-2	3,4

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

ASSAY HANDLING

The RVEV assay 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: Primer/Probe mix (5X) for Rhinoviruses, Enteroviruses, and hRPP30DNA.

Tube 2: (**Do NOT add to specimen unknowns**) Positive control: Synthetic 500 nt. DNA fragments of Rhinovirus type C and hRPP30DNA.

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 (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.
For 10 µL reactions, divide all of the amounts above by a factor of 2.

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

Step	Thermocycling Protocol:
1	Incubate @ 50 °C for 10 minutes
2	Incubate @ 95 °C for 3 minutes
3	Incubate @ 95 °C for 5 seconds
4	Incubate @ 55 °C for 22 seconds
5	Plate Read
6	Go to Step 2, repeat 44× more

NOTES

[1] FAM™ (Carboxyfluorescein), a trademark of Life Technologies Corporation.

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

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

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

[5] TaqMan™ is a trademark of Roche Diagnostics, Inc.

RESULT INTERPRETATION

After running the qPCR reaction, use the instrument software to determine the quantification cycle, C_q (or use C_T if your instrument does not have the capability to compute a C_q). Fluorescence channels with a C_q < 38 cycles, and final RFU >Threshold are 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 Pro with 96 well plate with 200 µL wells and 20 µL reaction volume, the average RFU was approximately 270,000 and we used a threshold of 27,000 for calling positives or "+" in the Table below.

Rhino/ Enteroviruses FAM™	hRPP30 TAMRA™	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 **Rhinovirus/Enterovirus** RNA. The sample may not contain human RPP30 DNA.
+	+	The sample contains **Rhinovirus/Enterovirus** RNA and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The RVEV assay verification was carried out as a 7-plex assay (2 colors) that simultaneously detects RNA from Rhinovirus types A, B, and C, Enteroviruses A71 and D68 (and other A and D types), Coxsackievirus type A and B, Poliovirus serotypes 1, 2, and 3, and human RPP30 DNA, which serves as a positive control assay.

Experiments were performed in triplicate using the experimental procedure given above, but with different RNA or DNA samples added to each reaction. The samples used for the verification experiments were RNA transcribed using Jena Bioscience High Yield T7 mRNA synthesis kit (#RNT-107-S) and human genomic DNA from Promega Corporation (#G3041). RNA was transcribed from synthetic 500 bp DNA constructs (from Twist Biosciences) harboring the regions of interest from the pathogen genomes. **Figure 1** shows the results of these experiments, which indicate that the 7-plex specifically detects the different pathogens.

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

Conclusion: The data in **Figure 1** indicate that the 7-plex primers and probes specifically detect the pathogens and are also compatible with RPP30_DNA positive control primers.

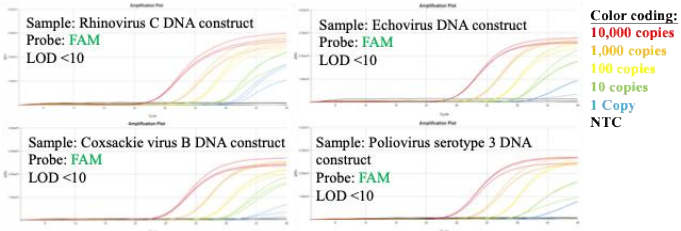

Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

Figure 1: Verification experiments with single and multiple 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. Continued on next page.

CONTACT US

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

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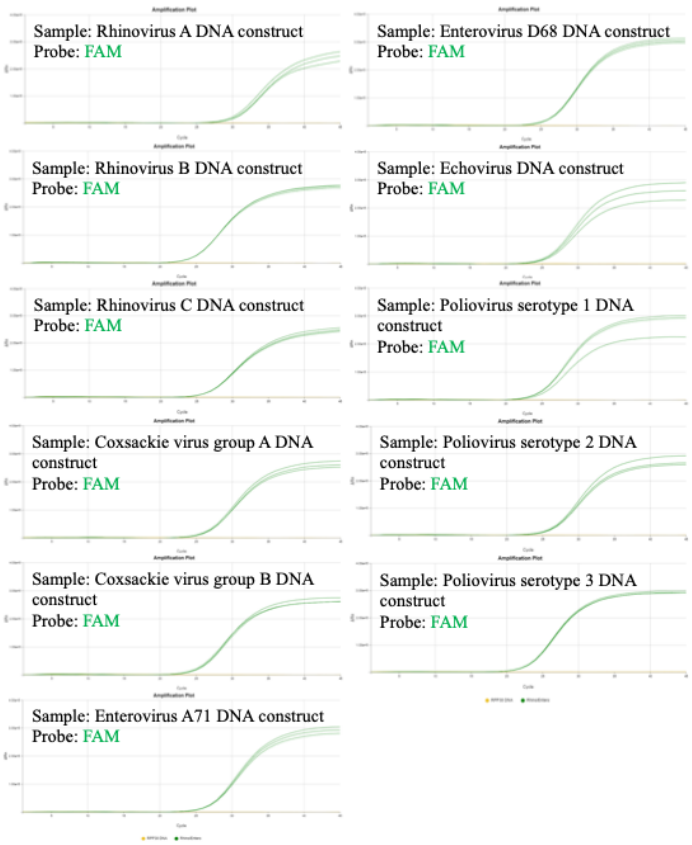

Figure 1 (continued): Verification experiments with single and multiple targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.