PCRassays.com

ASSAY NAME: RVEV_R_BR
(Rhino/Enteroviruses, RNA control, for BioRad)

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 types 1, 2, and 3, and human RPP30 RNA

SKU:
BUN-RVEV-R-BR-100 (Bio-Rad RNA control)

(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 (BioRad, QuantStudio). The verification data presented in this PIS were performed using BUN-RVEV-R-BR-100 (i.e., using an RNA control) 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.

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 types 1, 2, and 3. This assay does not detect Parechoviruses. All of these pathogens are detected with a single FAM labelled probe but with a mixture of 7 primer sets to amplify all the different types. The assay also detects a human extraction control (either endogenous human RPP30 DNA or spike-in RPP30 RNA).

Table of Dyes used in this assay:

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

The probes are designed as TaqMan[4] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity.

ASSAY HANDLING

The RVEV assay is shipped at ambient temperature, 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 hRPP30RNA.

Tube 2: (**Do NOT add to specimen unknowns**) Positive control: 5000 copies/µl of transcribed 500 nt. RNA fragments of Rhinovirus type C and hRPP30RNA.

Tube 3: (optional) Spike-in RNA control. 1.0E6 copies/uL of transcribed 500 nt. region of spliced human RPP30 RNA.

Tube 4: 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

NOTE for the RPP30-RNA version of the kit (BUN-RVEV-R-BR-100): Most samples typically do NOT contain enough undegraded human RNA to detect; thus, the user should add 1 µL of spike-in control (Tube 3) to each specimen before extraction. **Do not add directly to the PCR reaction!** It serves as extraction, RT, and PCR reaction control. For the RPP30-DNA version of the kit (BUN-RVEV-D-BR-100), no spike-in is needed because most human samples contain sufficient genomic DNA to detect.

Perform nucleic acid extraction/purification (recommended).

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

Component	Volume (µL)
InhibiTaq 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.

A PCR protocol was used for verification on a BioRad CFX96 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 15 seconds
5	Plate Read
6	Go to Step 2, repeat 44× more

NOTES

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

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

[3] HEX™ (Hexachloro-fluorescein) trademark of ThermoFisher Sci.

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

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 200 on BioRad instruments.

Rhino/ Enteroviruses FAM™	hRPP30 HEX™	Recommended Interpretation
–	–	The PCR reaction failed. Please repeat the experiment
–	+	The sample does not contain pathogen of interest. The sample contains human RPP30 RNA.
+	–	The sample contains **Rhinovirus/Enterovirus** RNA. The sample may not contain human RPP30 RNA.
+	+	The sample contains **Rhinovirus/Enterovirus** RNA and human RPP30 RNA.

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, Coxsackievirus type A and B, Poliovirus types 1, 2, and 3, and human RPP30 RNA, which serves as a positive 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 were RNA transcribed using Jena Bioscience High Yield T7 mRNA synthesis kit (#RNT-107-S). 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 dilution series only target RNA 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_RNA positive control primers.

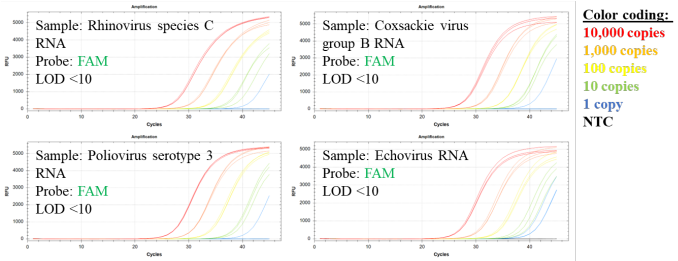

Figure 2: Serial dilution experiments (using BUN-RVEV-R-BR-100) show LOD <10 molecules for the transcribed RNA of each target.

CONTACT US

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

Address: Michigan Life Science and Innovation Center,
46701 Commerce Center Dr, Plymouth, MI 48170
Phone: (734) 222-9080

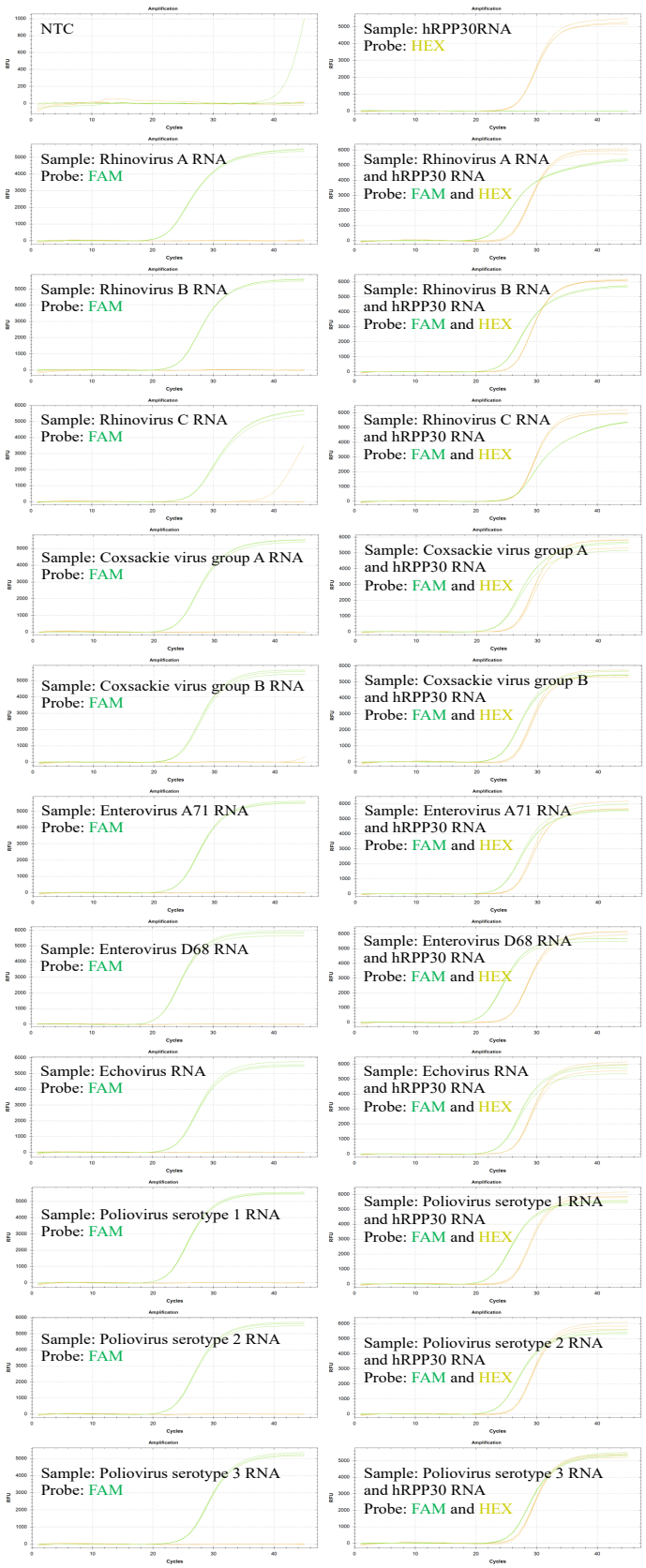

Figure 1: Verification experiments (using BUN-RVEV-R-BR-100) with single and multiple targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.