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PCRassays.com

Assay: VURP5_BR (Viral URP5 for BioRad)

Quantity: 100 x 20µL PCR reactions

5-plex assay: SARS-CoV-2, Influenza A, Influenza B, Respiratory syncytial virus A and B, and human RPP30 RNA

SKU: PNP-VURP5-D-BR-100 (BioRad)
PNP-VURP5-R-BR-100 (BioRad)

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

SCOPE OF THIS DOCUMENT:

The oligonucleotide recipes are optimized for each instrument (Bio-Rad, QuantStudio, MIC). The verification data presented in this product information sheet were performed using PNP-VURP5-D-BR-100 on a Bio-Rad 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 VURP5 assay are provided in Tube 1 as a 5X concentrated working solution that detects 4 pathogens and a human extraction control.

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
Flu A	FAM	BHQ-1	1,2
RPP30-RNA control	HEX	BHQ-1	7
SARS-CoV-2	TEX615	BHQ-2	3,4
RSV (A and B)	Cy5	BHQ-2	5
Flu B	Cy5.5	BHQ-2	6

The probes are designed as TaqMan[8] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity.

ASSAY HANDLING AND CONTAMINATION

The VURP5 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.

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

Assay contents:

Tube 1: Primer/Probe mix (5X) for SARS-CoV-2, Flu A, Flu B RSV A/B, and hRPP30RNA.

Tube 2: (**Do NOT add to specimen unknowns**) Positive control: 5000 copies/µl positive controls of synthetic 500 bp DNA fragments of Flu A, SARS-CoV-2, RSVA/B, Flu B, and human RPP30RNA.

Tube 3: Spike-in control. See note in the Experimental section about DNA vs. RNA control. For PNP-VURP5-**R**-BR-100: 1.0E6 copies/uL of transcribed 500 nt. **RNA** region of human RPP30 mRNA.

Tube 4: InhibiTaq 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: The VURP-D version of the assay will detect endogenous human DNA (RPP30 gene) in the sample. Typical samples contain enough human DNA so that it is not necessary to add synthetic DNA to reactions. In contrast, most samples typically do NOT contain enough undegraded human RNA to detect; thus, for VURP-R version of the assay 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 and PCR reaction control.

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	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 Bio-Rad CFX96, 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 3, repeat 44x more

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 > 200 (BioRad instruments) is considered "positive" or "+" in the Table below.

Flu A FAM™	SARS-CoV-2 TEX615™	RSVA/B Cy5™	Flu B Cy5.5™	RPP30 HEX™	Recommended Interpretation
–	–	–	-	–	The PCR reaction failed. Please repeat the experiment.
–	–	–	-	+	The sample contains human RPP30 RNA. The sample doesn't contain bacterial RNA.
+	–	–	-	–	The sample contains Flu A RNA. The sample may not contain human RPP30 RNA.
+	–	–	-	+	The sample contains Flu A RNA and human RPP30 RNA.
–	+	–	-	–	The sample contains SARS-CoV-2 RNA. The sample may not contain human RPP30 RNA.
–	+	–	-	+	The sample contains SARS-CoV-2 RNA and human RPP30 RNA.
–	–	+	-	–	The sample contains RSV RNA. The sample may not contain human RPP30 RNA.
–	–	+	-	+	The sample contains RSV RNA and human RPP30 RNA.
-	-	-	+	-	The sample contains Flu B RNA. The sample may not contain human RPP30 RNA.
-	-	-	+	+	The sample contains Flu B RNA and human RPP30 RNA.
+	+	+	+	–	The sample contains Flu A RNA, SARS-CoV-2 RNA, RSV RNA, and Flu B RNA.The sample may not contain human RPP30 RNA.
+	+	+	+	+	The sample contains Flu A RNA, SARS-CoV-2 RNA, RSV RNA, Flu B RNA, and human RPP30 RNA.

VERIFICATION EXPERIMENTS

The VURP5 assay verification was carried out as a 5-plex assay, which simultaneously detects RNA from Influenza A, SARS-CoV-2, Respiratory syncytial virus, Influenza B, 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 contained 1×10^4 copies/reaction of synthetic 500 bp DNA constructs (from Twist Biosciences) harboring the regions of interest from the pathogen genomes, human RPP30 RNA gene, full-length synthetic SARS CoV2 RNA (Twist – control 50, Omicron variant), quantitative genomic RNA obtained from ATCC: FluA H1N1 (VR-1894DQ), FluA H3N2 (VR-1882DQ), RSVA (VR-1540DQ), RSVB (VR-1803D), Flu B (VR-1804DQ), and Total human brain RNA. **Figure 1** shows the results of these experiments, which indicate that the 5-plex specifically detects the different pathogens.

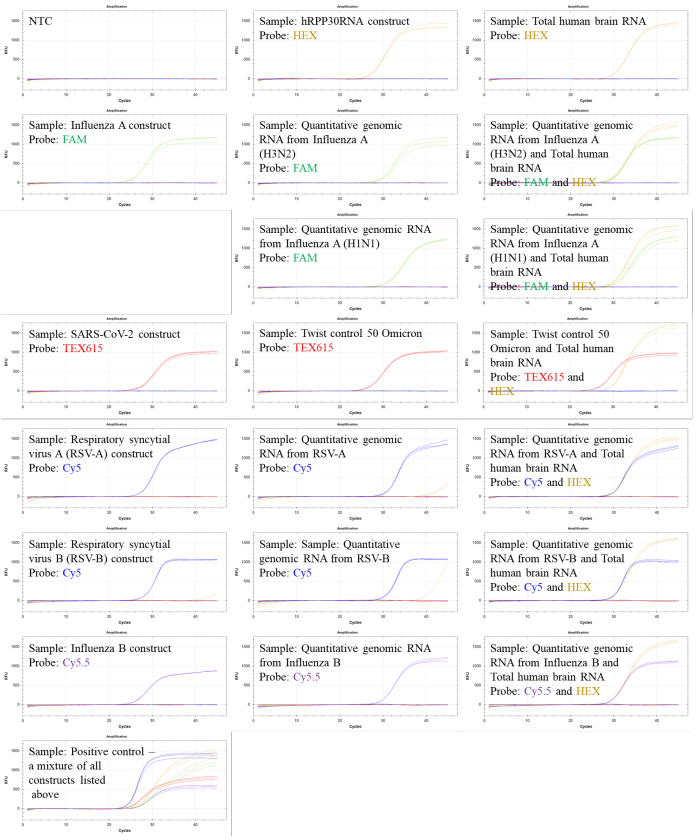

Figure 1: Verification experiments with single and multiple targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

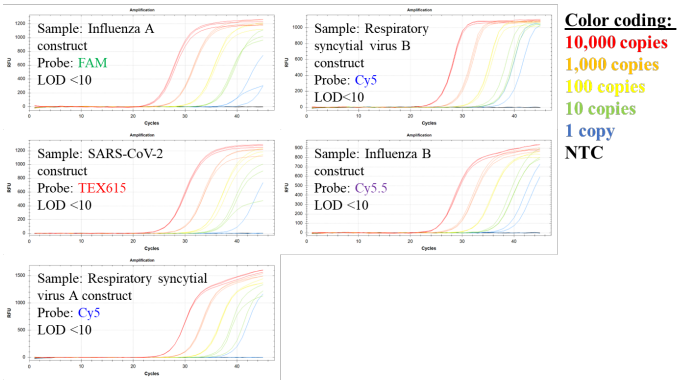

Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic RNA construct of each target.

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 5-plex primers and probes specifically detect and differentiate the pathogens and are also compatible with RPP30 RNA positive control primers. Human genomic RNA doesn't interfere with the detection of the pathogens.

NOTES

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

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

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

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

[5] Cy5™, a trademark of GE Healthcare.

[6] Cy5.5, a trademark of GE Healthcare.

[7] HEX™ (Hexachloro-fluorescein), a trademark of Thermo Fisher Scientific.

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

CONTACT US

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

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