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Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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PCRassays.com

ASSAY NAME: VURP7 (4-color)

Quantity: 100 x 20µL PCR reactions

6-plex (4-color) assay: SARS-CoV-2, Influenza types A and B, Respiratory syncytial virus types A and B, and human RPP30 RNA

SKU: PNP-VURP7-D-BR-100 (Bio-Rad)
PNP-VURP7-D-QS-100 (QuantStudio)
PNP-VURP7-D-MIC-100 (BMS MIC)

(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 pre-validation data presented in this PIS were performed using PNP-VURP7-D-MIC-100 on a BMS MIC instrument and on a BioRad CFX96 instrument. 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 VURP7 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 and Flu B	FAM	BHQ-1	1,2
RPP30-DNA control	HEX	BHQ-1	6
SARS-CoV-2	TexRED615	BHQ-2	3,4
RSV A and RSV B	Cy5	BHQ-2	5

The probes are designed as TaqMan[7] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity.

ASSAY HANDLING AND CONTAMINATION

The VURP7 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 targets: SARS-CoV-2, Flu A, Flu B, RSV A, RSV 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 SARS-CoV-2, Flu A, Flu B, RSV A, RSV B.

Tube 3: (Optional) RNA Spike-in control. 1.0E6 copies/uL of synthetic transcribed 500 nucleotide 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

(Mandatory) Typical upper respiratory samples contain very little human mRNA, and thus it is important to 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.

Perform nucleic acid extraction/purification (recommended). **Note: molecular biology grade water (NOT included) should be used to prepare the PCR rxns.**

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

Component	Volume (µL)
InhibiTaq Standard 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 BMS MIC and BioRad CFX96 instruments, 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 44× more

For QuantStudio instruments, we recommend a Step 4 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.

FluA & FluB FAM™	SARS-CoV-2 TexRED615™	RSV A & B Cy5™	RPP30 HEX™	Recommended Interpretation
–	–	–	–	The PCR reaction failed. Please repeat the experiment.
–	–	–	+	The sample contains human RPP30 RNA. The sample doesn't contain viral RNA.
+	–	–	–	The sample contains Flu A or Flu B. The sample may not contain human RPP30 RNA.
+	–	–	+	The sample contains Flu A or Flu B 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 A or RSV B RNA. The sample may not contain human RPP30 RNA.
–	–	+	+	The sample contains RSV A or RSV B RNA and human RPP30 RNA.
+	+	+	–	The sample contains Flu A or Flu B RNA, SARS-CoV-2, and RSV A or RSV B RNA. The sample may not contain human RPP30 RNA.
+	+	+	+	The sample contains Flu A or Flu B RNA, SARS-CoV-2, and RSV A or RSV B RNA, and human RPP30 RNA.

VERIFICATION EXPERIMENTS

The VURP7 assay verification was carried out as a 6-plex assay (4 colors), which simultaneously detects DNA from SARS-CoV-2, Influenza A, Influenza B, Respiratory syncytial virus types A and 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, and human RPP30 RNA gene. RNA samples were from Twist Biosciences (SARS-CoV-2 RNA control 50), and quantitative genomic RNA extracts from ATCC (Flu A H1N1, Flu A H3N2, Flu B, RSV A, RSV B). **Figure 1** shows the results of these experiments, which indicate that the 6-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 construct was added. The results show a limit of detection (LOD) <10 copies/reaction.

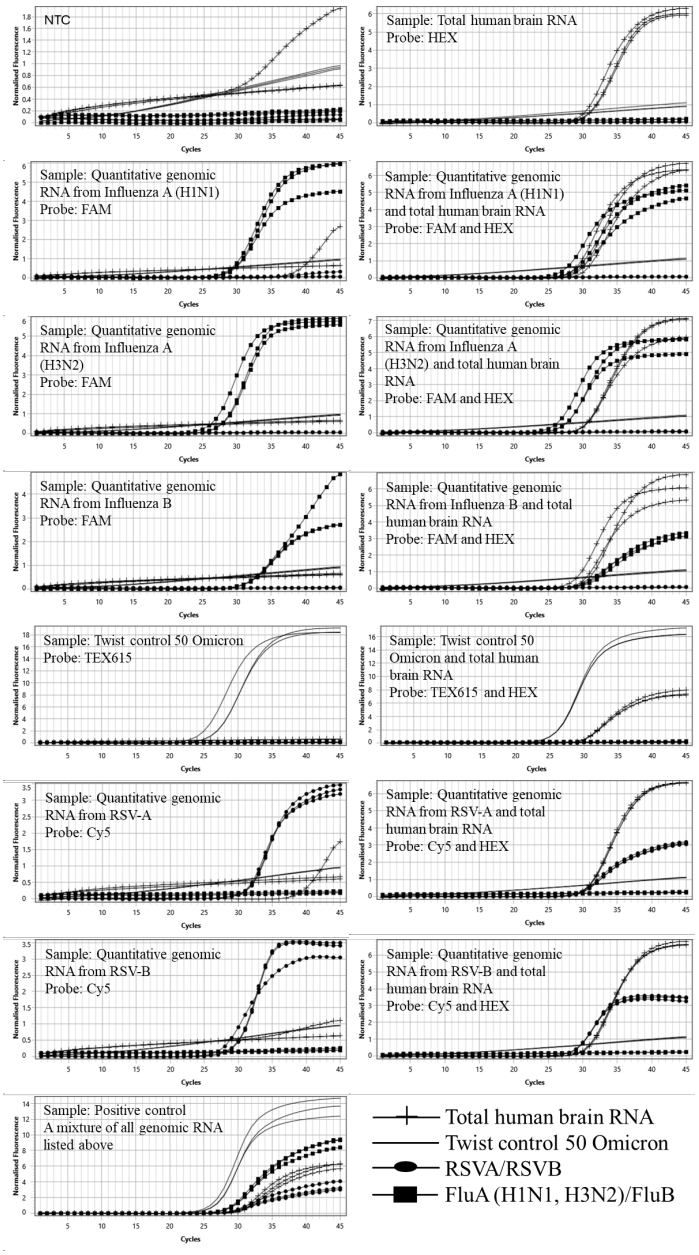

Figure 1: Verification experiments (performed on BMS MIC instrument) with single and multiple targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification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

Conclusion: The data in **Figure 1** indicate that the 6-plex primers and probes specifically detect and differentiate the pathogens and are also compatible with RPP30 positive control primers. Human genomic DNA doesn't interfere with the detection of the pathogens.

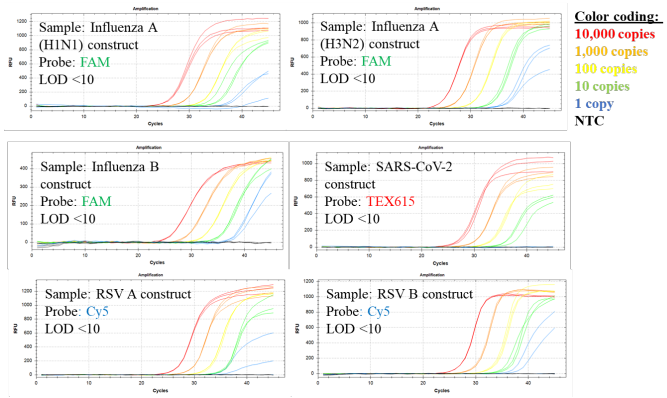

Figure 2: Serial dilution experiments (performed on BioRad CFX96 instrument) show LOD <10 molecules for the synthetic DNA construct of each target.

CONTACT US

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

Address: Michigan Life Science and Innovation Center,
46701 Commerce Center Dr, Plymouth, MI 48170

Phone: (734) 222-9080

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

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