

ASSAY NAME: PIV-D-QS
Quantity: 100 x 20µL PCR reactions
5-color assay Human parainfluenza viruses 1, 2, 3, 4a, 4b, and human RPP30 DNA
SKU: BUN-PIV-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 (BioRad, QuantStudio, MIC). The verification data presented in this PIS were performed using BUN-PIV-D-QS-100 on a QuantStudio™ 7 Flex Real-Time 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 PIV-D assay are provided in Tube 1 as a 5X concentrated working solution that detects human parainfluenza viruses (PIV) 1, 2, 3, 4a, and 4b. The assay also detects a human extraction control (either endogenous human RPP30 DNA or spike-in RPP30 DNA).

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
PIV4a and 4b	FAM	BHQ-1	1,2
PIV3	HEX	BHQ-1	3
RPP30-DNA control	TAMRA	BHQ-2	4, 5
PIV1	CalFluor610	BHQ-2	6
PIV2	Cy5	BHQ-2	7

The probes are designed as TaqMan⁸ cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity.

ASSAY HANDLING

The PIV-D assay is shipped with frozen cold packs, 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 PIV 1, 2, 3, 4a, 4b, and hRPP30DNA.

Tube 2: (Do NOT add to specimen unknowns) Positive control: 5000 copies/µl of DNA constructs of PIV 1, 2, 3, 4a, 4b, 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 reactions (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 QuantStudio™ 7 Flex 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

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 >200,000 on QuantStudio 5, 6, 7, 12K instruments is considered “positive” or “+” in the Table below.

PIV4a & 4b FAM™	PIV3 HEX™	hRPP30 TAMA™	PIV1 CalFluor 610™	PIV2 Cy5™	Recommended Interpretation
—	—	—	—	—	The PCR reaction failed. Please repeat the experiment
—	—	+	—	—	The sample does not contain DNA of interest. The sample contains human RPP30 DNA.
+	—	—	—	—	The sample contains PIV4 DNA. The sample may not contain human RPP30 DNA.
+	—	+	—	—	The sample contains PIV4 DNA and human RPP30 DNA.
—	+	—	—	—	The sample contains PIV3 DNA. The sample may not contain human RPP30 DNA.
—	+	+	—	—	The sample contains PIV3 DNA and human RPP30 DNA.
—	—	—	+	—	The sample contains PIV1 DNA. The sample may not contain human RPP30 DNA.
—	—	+	+	—	The sample contains PIV1 DNA and human RPP30 DNA.
—	—	—	—	+	The sample contains PIV2 DNA. The sample may not contain human RPP30 DNA.
—	—	+	—	+	The sample contains PIV2 DNA and human RPP30 DNA.
+	+	—	+	+	The sample contains PIV4, PIV3, PIV1, and PIV2 DNA. The sample may not contain human RPP30 DNA.
+	+	+	+	+	The sample contains PIV4, PIV3, PIV1, and PIV2 DNA and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The PIV-D assay verification was carried out as a 5-plex assay that simultaneously detects RNA from human PIV1, 2, 3, 4a, 4b, and human RPP30 DNA, which serves as a positive control.

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, standard RNA samples, purchased from ATCC (PIV2 #VR-92D and PIV4b #VR-1377D) and RNA extracted from patients generously provided by Assurance Scientific Lab (PIV 1, 3, and 4a). The human genomic DNA (Sigma-Aldrich 11691112001) and total human brain RNA (Takara 636530) were used to create a matrix to mimic DNA/RNA extractions from human samples.

Figure 1 shows the results of these experiments, which indicate that the 5-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 5-plex primers and probes specifically detect the pathogens and are also compatible with RPP30 DNA positive control primers within a sample matrix composed of both human DNA and RNA.

NOTES

- 1 FAM™ (Carboxyfluorescein), a trademark of Life Technologies Corp.
- 2 BHQ-1™ (Black Hole Quencher) is a trademark of Biosearch Tech., Inc.
- 3 HEX™ (Hexachloro-fluorescein), a trademark of Thermo Fisher Scientific.
- 4 TAMRA (Carboxyltetramethylrhodamine) is a trademark of Applera Cor.
- 5 BHQ-2™ (Black Hole Quencher) is a trademark of Biosearch Tech., Inc.
- 6 CalFluor610™ is a trademark of Biosearch Technologies, Inc.
- 7 Cy5™, a trademark of GE Healthcare.
- 8 TaqMan™ is a trademark of Roche Diagnostics, Inc.

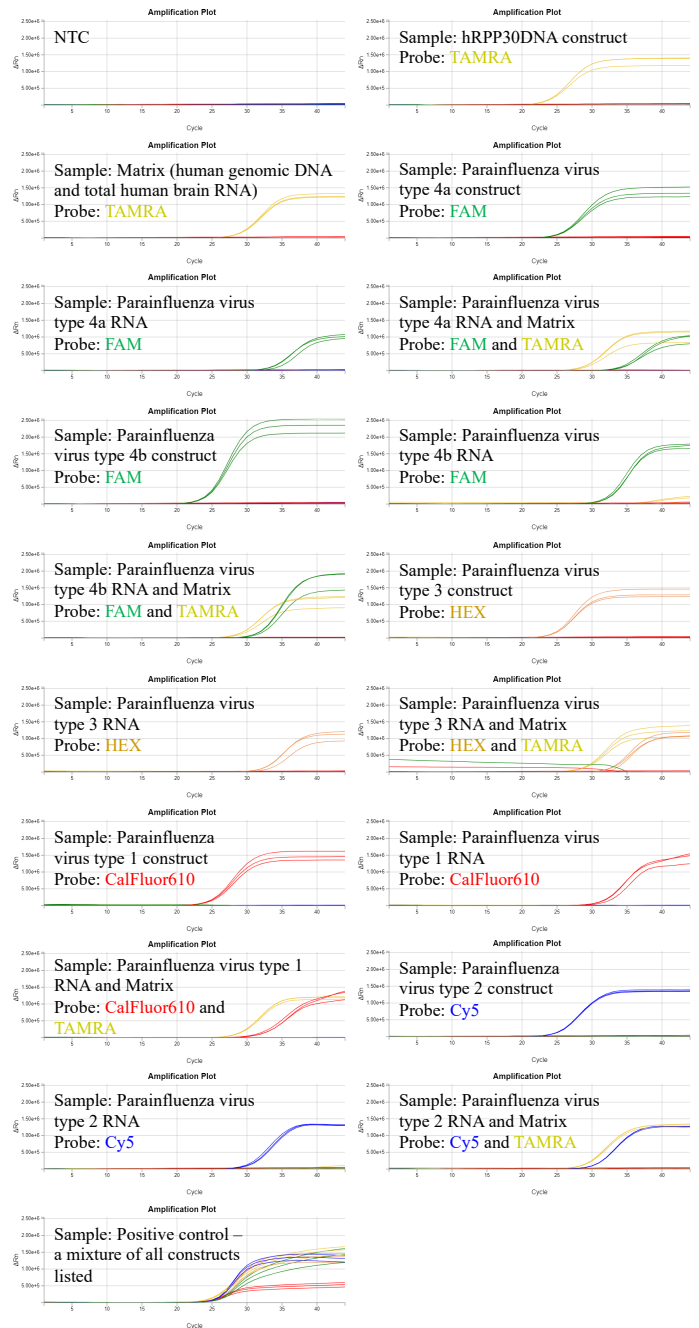


Figure 1: Verification experiments with single and multiple targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

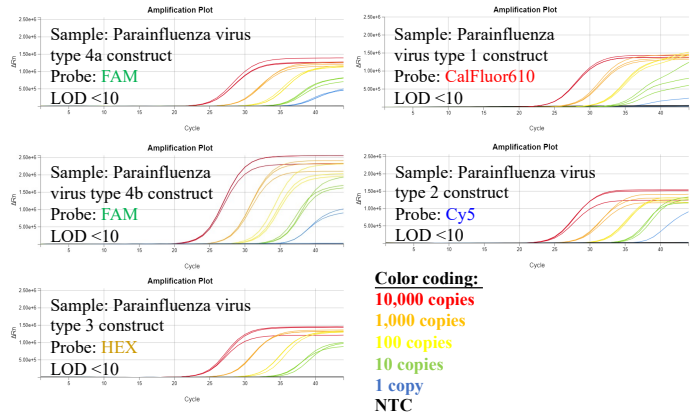


Figure 2: Serial dilution experiments show LOD <10 molecules for the transcribed RNA of each target.

CONTACT US

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

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