PCRassays.com

ASSAY NAME: HPN3 (Herpes Panel 3)

Quantity: 100 x 20µL PCR reactions

4-plex assay: Human herpesvirus type 6 (Roseola virus), Human herpesvirus type 4 (Epstein-Barr virus), Human herpesvirus type 8 (Kaposi's sarcoma virus), and human RPP30 DNA

SKU: PNP-HPN3-D-BR-100 (Bio-Rad)

(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 (Bio-Rad, QuantStudio). The verification data presented in this PIS were performed using PNP-HPN3-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 are planning to use a different qPCR instrument.

CONTENTS

The primers and probes in the HPN3 assay are provided in Tube 1 as a 5X concentrated working solution that detects 3 pathogens and a human extraction control.

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
HHV-6	FAM	BHQ-1	1, 2
RPP30-DNA control	HEX	BHQ-1	3
HHV-4	CalFluor610	BHQ-2	4, 5
HHV-8	Cy5.5	BHQ-2	6

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 HPN3 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.

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: 5X Primer/Probe mix for HHV-6, HHV-4, HHV-8, and hRPP30DNA.

Tube 2: (**Do NOT add to specimen unknowns**) Positive control: 5000 copies/µl positive controls of synthetic 500 bp DNA fragments of HHV-6, HHV-4, HHV-8, and human RPP30DNA.

Tube 3: InhibiTaq Standard 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).

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

For BioRad instruments we strongly recommend the use of "white plates" to maximize fluorescence detection (HSP-9655, Hard-Shell 96-well skirted plates with white shell and white wells). Set up your PCR reaction (20 µL) as follows on ice:

Component	Volume (µL)
InhibiTaq qPCR enzyme mastermix (2X)	10
Primer/Probe mix (5X)	4
Sample or Positive Control	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 system using white plates, with the following program:

Step	Thermocycling Protocol:
1	Incubate @ 95 °C for 2 minutes
2	Incubate @ 95 °C for 3 seconds
3	Incubate @ 55 °C for 15 seconds
4	Plate Read
5	Go to Step 2, repeat 44× more

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). PCR reactions are considered "positive" or "+" in the table below if the RFU > "threshold" and the Cq <38 cycles for HEX, CalFluor610, and Cy5.5 channels or Cq <36 cycles for the FAM channel. [See note 8] 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 Bio-Rad CFX-96 with 100 µL wells and 20 µL reaction volume, the average RFU was approximately 2,500, we used a threshold of 200 for calling positives or "+" in the Table below.

HHV-6 FAM™	HHV-4 CalFluor 610™	HHV-8 Cy5.5™	RPP30 HEX™	Recommended Interpretation
–	–	–	–	The PCR reaction failed. Please repeat the experiment.
–	–	–	+	The sample contains human RPP30 DNA. The sample doesn't contain bacterial DNA.
+	–	–	–	The sample contains HHV-6 DNA. The sample may not contain human RPP30 DNA.
+	–	–	+	The sample contains HHV-6 DNA and human RPP30 DNA.
–	+	–	–	The sample contains HHV-4 DNA. The sample may not contain human RPP30 DNA.
–	+	–	+	The sample contains HHV-4 DNA and human RPP30 DNA.
–	–	+	–	The sample contains HHV-8 DNA. The sample may not contain human RPP30 DNA.
–	–	+	+	The sample contains HHV-8 DNA and human RPP30 DNA.
+	+	+	–	The sample contains HHV-6 DNA, HHV-4 DNA, and HHV-8 DNA. The sample may not contain human RPP30 DNA.
+	+	+	+	The sample contains HHV-6 DNA, HHV-4 DNA, HHV-8 DNA, and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The HPN3 assay verification was carried out as a 4-plex assay, which simultaneously detects DNA from Human herpesvirus type 6, Human herpesvirus type 4, Human herpesvirus type 8, and human RPP30 DNA, which serves as a positive extraction-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 DNA gene, and human genomic DNA. **Figure 1** shows the results of these experiments, which indicate that the 4-plex specifically detects the different viral species in the human genomic DNA matrix.

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

NOTES

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

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

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

[4] CalFluor610™ is a trademark of Biosearch Technologies, Inc.

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

[6] Cy5.5™ is a trademark of GE Healthcare.

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

[8] Clinical observations must be used along with positive test results to determine if the infection is latent or active. "By adulthood, more than 95% of the population is seropositive for HHV-6A, HHV-6B, or both variants." King, O. (2023, August 8). Herpes virus type 6. StatPearls. https://www.ncbi.nlm.nih.gov/books/NBK540998/

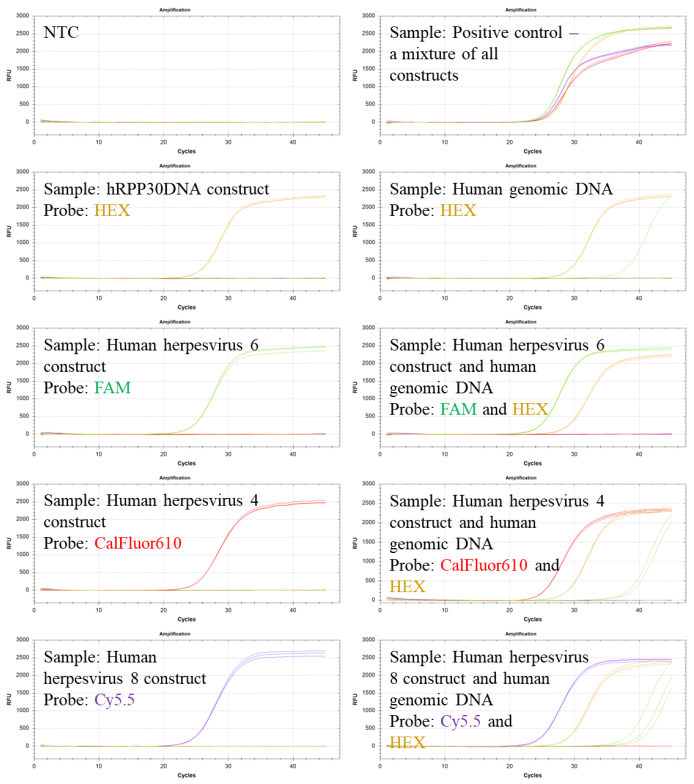

Figure 1: Verification experiments with single and multiple targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific. [See note 8]

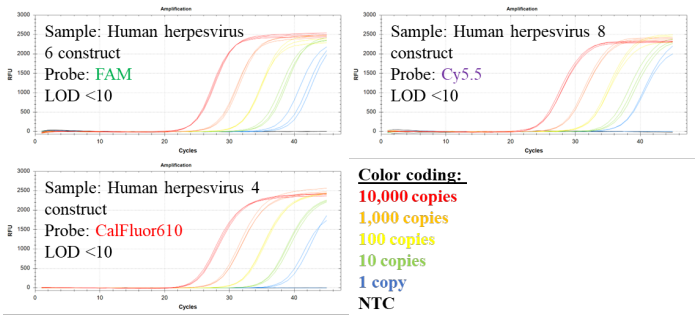

Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

Conclusion: The data in **Figure 1** indicate that the 4-plex primers and probes specifically detect and differentiate the pathogens and are also compatible with RPP30 DNA positive control primers. Human genomic DNA doesn't interfere with the detection of the pathogens.

CONTACT US

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

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