ASSAY NAME: HHV6

Quantity: 100 x 20µL PCR reactions

2-plex assay: HHV6 and human RPP30 DNA

SKU#:
BUN-HHV6-D-QS (QuantStudio with control assay)

(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 verification data presented in this document were performed using PNP-HPN3-D-QS-100 on a QuantStudio 7 Flex. 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 HHV6 assay are provided in Tube 1 as a 20X concentrated working solution that detects HHV6 and a human control.

Table of Dyes used in this kit:

Pathogen/Target	Dyes	Quencher	Refs.
HHV-6	**FAM**	BHQ-1	1, 2
RPP30-DNA control	**TAMRA**	BHQ-2	3, 4

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

KIT HANDLING AND CONTAMINATION

The HHV6 bundle is shipped on ice, 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: 20X Primer/Probe mix for HHV6 and hRPP30DNA

Tube 2: (Do NOT add to specimen unknowns) Positive control: Synthetic 500 bp DNA fragments of HHV6 and hRPP30DNA.

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).

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

Component	Volume (µL)
InhibiTaq Standard qPCR enzyme mastermix (2X)	10
Primer/Probe mix (20X)	1
Sample	2
Molecular biology grade water (Not included)	7

Notes: To improve assay sensitivity, up to 9 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. Molecular biology grade water should be used to prepare the PCR reactions, which is NOT included in this kit.

A PCR protocol was used for verification on a QuantStudio 7 Flex, 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 22 seconds
4	Plate Read
5	Go to Step 2, repeat 44xmore

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.

HHV-6 FAM™	hRPP30 TAMRA™	Recommended Interpretation
–	–	The PCR reaction failed. Please repeat the experiment
–	+	The sample does not contain HHV6 viral DNA. The sample contains human RPP30 DNA.
+	–	The sample contains HHV6 DNA. The sample may not contain human RPP30 DNA. Site 2 of HAV may contain a mutation.
+	+	The sample contains HHV6 DNA and human RPP30 DNA. Site 2 of HAV may contain a mutation.

VERIFICATION EXPERIMENTS

The HHV6 primers and probes were previously verified as part of the study of a 4-plex assay (SKU#: PNP-HPN3-D-QS-100), which simultaneously detects DNA from human herpesvirus types 4, 6, 8, and human RPP30 DNA, which serves as a positive extraction-control assay.

Experiments were performed in triplicate on a QuantStudio 7 using the experimental procedure given above, but with different samples added to each reaction. The samples used for the verification experiments contained 1x10^4 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.

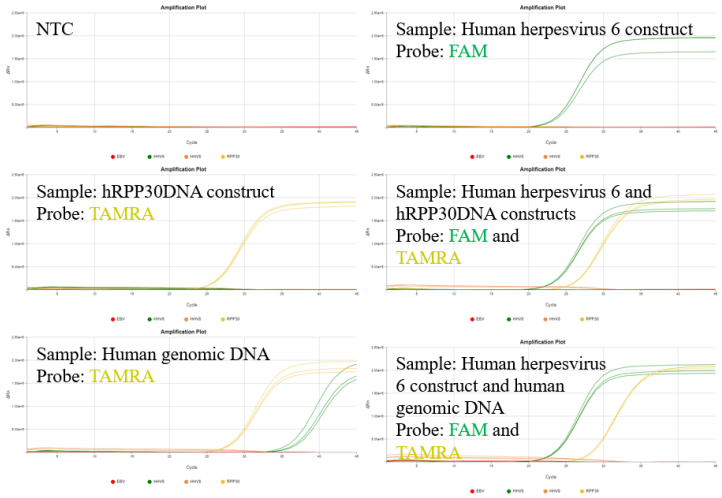
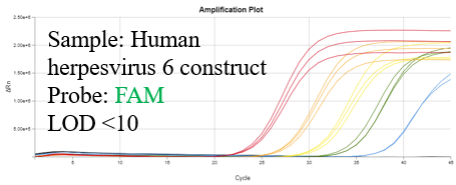


Figure 1: Verification experiments with single or double target(s) (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

The limit of detection (LOD) was estimated by performing serial dilution experiments (**Figure 2)** used samples with 1×10^4 copies/reaction of transcribed 500 nt.

Figure 2: Serial dilution experiments show LOD <10 molecules.



Color coding:
10,000 copies
1,000 copies
100 copies
10 copies
1 copy
NTC

Conclusion: The data in **Figures 1 and 2** indicate that the HHV6 primers and probes are compatible with DNAS RPP30 DNA positive control primers and probe in the human genomic DNA matrix.

CONTACT US

For assistance, please contact DNA Software using the link: https://dnasoft.jira.com/servicedesk/customer/portals

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NOTES

[1] FAM™ (Carboxyfluorescein), a trademark of Life Technologies Corporation
[2] BHQ-1™ (Black Hole Quencher) is a trademark of Biosearch Tech., Inc.
[3] TAMRA (Carboxyltetramethylrhodamine) is a trademark of Applera Cor.
[4] BHQ-2™ (Black Hole Quencher) is a trademark of Biosearch Tech., Inc.
[5] TaqMan™ is a trademark of Roche Diagnostics, Inc.