Kit: Herpes Panel 1
Quantity: 100 x 20µL PCR reactions

5-plex assay: Cytomegalovirus, Herpes simplex virus type 2, Varicella zoster virus, Herpes simplex virus type 1, and human RPP30 DNA

SKU: PNP-HPN1-D-BR-100 (Bio-Rad)
PNP-HPN1-D-QS-100 (QuantStudio)

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

Kit contents:

Tube 1: 5X Primer/Probe mix for CMV, HSV-2, VZV, HSV-1, and hRPP30DNA

(Optional) Tube 2: 5000 copies/µl Positive controls of synthetic 500 bp DNA for CMV, HSV-2, VZV, HSV-1, and human RPP30DNA.

SCOPE OF THIS IFU:

For the Bio-Rad CFX instruments use SKU: PNP-HPN1-D-BR-100 (5-plex assay including the human RPP30-DNA control assay). For the QuantStudio instruments use SKU: PNP-HPN1-D-QS-100. The recipes are optimized for each instrument (BioRad and QuantStudio). The pre-validation data for PNP-HPN1-D-BR-100 on a Bio-Rad CFX 96 are presented in this IFU. 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 Herpes Panel 1 (HPN1) kit are provided in Tube 1 as a 5X concentrated working solution that target 4 pathogens and a human control.

Table of Dyes used in this kit:

Pathogen/Target	Dyes	Quencher	Refs.
CMV	FAM	BHQ-1	1,2
RPP30-DNA control	HEX	BHQ-1	7
HSV-2	CalFluor-Red610	BHQ-2	3,4
VZV	Cy5	BHQ-2	5
HSV-1	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 (we recommend InhibiTaq PLUS qPCR Master Mix, from Empirical Biosciences, Cat number: ITMP-MM-2500).

Tube 2 contains a mixture of synthetic 500 bp DNA constructs containing the amplicon regions of all 4 pathogens and human RPP30DNA (i.e. positive extraction control). The concentration of each DNA construct is approximately 5,000 copies/µL. The Control DNA constructs are for validation purposes only. **Tube 2 should NOT be added to wells for specimen unknowns**.

KIT HANDLING AND CONTAMINATION

The Herpes Panel 1 kit is shipped at ambient temperature, and should be stored at -20 °C. The kit 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, which is NOT included in this kit.

EXPERIMENTAL

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

Component	Volume (µL)
InhibiTaq enzyme mastermix (2X)	10
Primer/Probe mix (5X)	4
Sample	2
Water	4

Note: The composition of this reaction is calculated based on the user manual of InhibiTaq PLUS qPCR MasterMix, from Empirical Biosciences. In a reaction with the positive control, 2 µL of the solution from Tube 2 should be added (i.e. the "sample").

A PCR protocol was used for pre-validation on a Bio-Rad CFX96™ Real-Time System, 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

For QuantStudio instruments, we recommend a Step 3 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 >200 is considered "positive" or "+" in the Table below. Note: On QuantStudio 5, 6, 7, 12K instruments the final RFU cutoff is >200,000.

CMV FAM™	HSV-2 TEX615™	VZV Cy5™	HSV-1 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 CMV DNA. The sample may not contain human RPP30 DNA.
+	–	–	–	+	The sample contains CMV DNA and human RPP30 DNA.
–	+	–	–	–	The sample contains HSV-2 DNA. The sample may not contain human RPP30 DNA.
–	+	–	–	+	The sample contains HSV-2 DNA and human RPP30 DNA.
–	–	+	–	–	The sample contains VZV DNA. The sample may not contain human RPP30 DNA.
–	–	+	–	+	The sample contains VZV DNA and human RPP30 DNA.
–	–	–	+	–	The sample contains HSV-1 DNA. The sample may not contain human RPP30 DNA.
–	–	–	+	+	The sample contains HSV-1 DNA and human RPP30 DNA.
+	+	+	+	–	The sample contains CMV DNA, HSV-2 DNA, VZV DNA, and HSV-1 DNA. The sample may not contain human RPP30 DNA.
+	+	+	+	+	The sample contains CMV DNA, HSV-2 DNA, VZV DNA, and HSV-1 DNA, and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The Herpes Panel 1 kit validation was carried out as a 5-plex assay, which simultaneously detects DNA from Cytomegalovirus, Herpes simplex virus type 2, Varicella zoster virus, Herpes simplex virus type 1, 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 5-plex specifically detects the different bacterial species.

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: Validation experiments with single 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. The **FAM** probe detects Cytomegalovirus DNA. The **TEX615** probe detects Herpes simplex virus type 2 DNA. The **Cy5** probe detects Varicella zoster virus DNA. The **Cy5.5** probe detects Herpes simplex virus type 1 DNA. The **HEX** probe detects human RPP30 DNA.

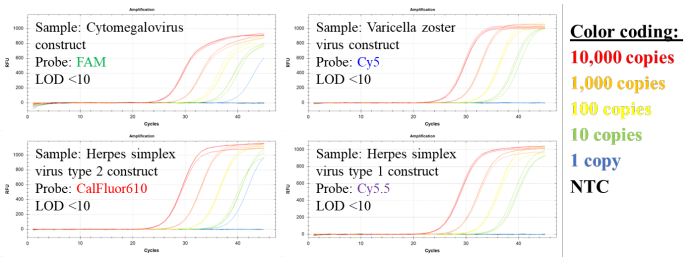

Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

Conclusion: The data in **Figure 1** indicate that the 5-plex primers and probes specifically detect and differentiate the virus types and are also compatible with RPP30_DNA positive control primers. Verifications of the four 2-plex kits (pathogen + control assays) (data not shown) indicate that human genomic DNA matrix doesn't affect detection of the pathogen DNA.

CONTACT US

For assistance, please contact DNA Software using the link:
DNA Software Help Center - Jira Service Management

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

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

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

[6] Cy5.5™ (Sulfo-Cyanine5.5) is a trademark of GE Healthcare.

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

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