PCRassays.com

ASSAY NAME: HSV2-D

Quantity: 100 x 20µL PCR reactions

Duplexed assay: Herpes simplex virus type-2 and human genomic RPP30 DNA

SKUs: PNP-HSV2-D-BR-100

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

ASSAY CONTENTS

Tube 1: 20X Primer/Probe mix for HSV2 and hRPP30DNA.

Tube 2: (Do NOT add to specimen unknowns) Positive control: Synthetic 500 bp DNA fragments for HSV2 and hRPP30DNA.

Tube 3: InhibiTaq 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.

SCOPE OF THIS DOCUMENT

The oligonucleotide recipes are optimized for each instrument (BioRad, QuantStudio, MIC). The verification data presented in this PIS were performed using PNP-HSV2-D-BR-100 on a BioRad CFX96. 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 HSV2 assay are provided in Tube 1 as a 20X concentrated working solution that detects the Herpes simplex virus type-1 genome and a human control.

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs
HSV2	**CALFluorRed610**	BHQ-2	1,2
RPP30-DNA Control	**HEX**	BHQ-1	3,4

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

ASSAY HANDLING

The HSV2 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.

Contamination should be avoided by using appropriate personal protective equipment (PPE), powder free gloves, aerosol barrier pipette tips, and a clean hood.

EXPERIMENTAL

Perform nucleic acid extraction/purification (recommended). Set up your reaction (20 µL) as follows on ice:

Component	Volume (µL)
InhibiTaq Enzyme mastermix (2X)	10
Primer/Probe mix (20X)	1
Sample	2
Water (Molecular Biology Grade)	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.

A PCR protocol was used in-house for verification 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 44xmore

RESULT INTERPRETATION

After running the qPCR reaction, use the instrument software to determine the quantification cycle, Cq (or use CT if your instrument does not have the capability to compute a Cq). Fluorescence channels with a Cq < 38 cycles, and final RFU > Threshold are considered "positive" or "+" in the Table below.

HSV2 (CALFluorRed610™)	RPP30 (HEX™)	Recommended Interpretation
–	–	The PCR reaction failed. Please repeat the experiment.
–	+	The sample doesn't contain Herpes simplex virus 2 DNA.
+	–	The sample contains Herpes simplex virus 2 DNA. The sample may not contain human RPP30 DNA.
+	+	The sample contains Herpes simplex virus 2 DNA. human RPP30 DNA.

VERIFICATION EXPERIMENTS

The HSV2-RPP30DNA kit validation was carried out as a duplexed assay, which simultaneously detects DNA from Herpes simplex virus 2 and DNA from the human RPP30DNA gene, 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 validation experiments contained 1×10^4 copies/reaction of synthetic 500 bp synthetic DNA constructs (from Twist Biosciences) harboring the regions of interest from Herpes simplex virus 2 genome and the RPP30DNA gene. The results of these experiments are shown in **Figure 1** below:

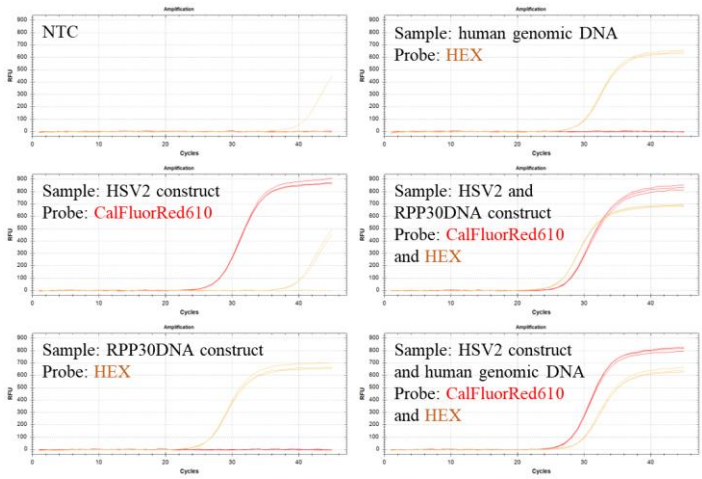

Figure 1: Validation experiments with single or double target(s) (given in text boxes for each panel). Both sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific. The **CALFluorRed610** probe detects Herpes simplex virus 2 construct DNA. The **HEX** probe detects RPP30DNA construct DNA.

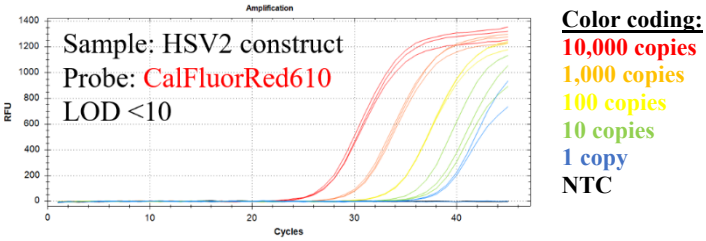

Figure 2: Serial dilution experiments show LOD <10 molecules for Herpes simplex virus 2 with 500 bp synthetic DNA construct.

Conclusion: The data in **Figure 1** indicate that the Herpes simplex 2 primers and probe are compatible with DNAS RPP30DNA positive control primers and probe in a 2-plex application to detect Herpes simplex virus 2 in the matrix of human sample extract.

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

CONTACT US

For assistance, please contact DNA Software, Inc. (the parent company of PCRassays.com) using the link:
https://www.dnasoftware.com/contact/

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

Phone: (734) 222-9080

NOTES

[1] CALFluorRed610™ is a trademark of Biosearch Technologies, Inc.

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

[3] HEX™ (Hexachloro-fluorescein), a trademark of Applera Corp.

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

[5] "TaqMan" is a trademark of Roche Molecular Systems, Inc.