PCRassays.com

ASSAY NAME: HEPV_BR384
(Hepatitis viruses for Bio-Rad 96 and 384)

Quantity: 100 x 20µL PCR reactions
4-plex assay: Hepatitis A, B, C, and human RPP30 DNA

SKU #: BUN-HEPV-D-BR384-100 (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 (QuantStudio, BioRad). The verification data presented in this PIS were performed using kit BUN-HEPV-D-BR384-100 on a Bio-Rad CFX96 using white-bottomed plates. This kit is compatible with both CFX-96 and CFX-384 instruments. 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 HEPV_BR384 assay are provided in Tube 1 as a 5X concentrated working solution that detects 3 pathogens, and a human control.

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
HCV	FAM	BHQ-1	1,2
RPP30-DNA control	HEX	BHQ-1	3
HAV	TEX615	BHQ-2	4,5
HBV	Cy5	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

The HEPV_BR384 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.

ASSAY CONTENTS:

Tube 1: 5X Primer/Probe mix for HAV, HBV, HCV, and hRPP30DNA.

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

Tube 3: InhibiTaq 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 PCR reaction (20 µL) as follows on ice:

Component	Volume (µL)
InhibiTaq 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.
For 10 µL reactions, divide all of the amounts above by a factor of 2.

A PCR protocol was used for verification on a Bio-Rad CFX96 system using white-bottomed plates, 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 20 seconds
5	Plate Read
6	Go to Step 3, repeat 43× more

RESULT INTERPRETATION

After running the qPCR reaction, use the instrument software to determine the quantification cycle, Cq (or use C_T if your instrument does not have the capability to compute a Cq). Fluorescence channels with a Cq < 38 cycles, and final RFU >Threshold is considered "positive" or "+" in the Table below. 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 BioRad CFX-96 with 100 µL wells and 20 µL reaction volume, the average RFU was approximately 1,800, we used a threshold of 180 for calling positives or "+" in the Table below.

HCV FAM™	hRPP30 HEX™	HAV TEX615™	HBV Cy5™	Recommended Interpretation
–	–	–	–	The PCR reaction failed. Please repeat the experiment
–	+	–	–	The sample does not contain pathogen RNA. The sample contains human RPP30 DNA.
+	–	–	–	The sample contains HCV RNA. The sample may not contain human RPP30 DNA.
+	+	–	–	The sample contains HCV RNA and human RPP30 DNA.
–	–	+	–	The sample contains HAV RNA. The sample may not contain human RPP30 DNA.
–	+	+	–	The sample contains HAV RNA and human RPP30 DNA.
–	–	–	+	The sample contains HBV DNA. The sample may not contain human RPP30 DNA.
–	+	–	+	The sample contains HBV DNA and human RPP30 DNA.
+	–	+	+	The sample contains HCV and HAV RNA, and HBV DNA. The sample may not contain human RPP30 DNA.
+	+	+	+	The sample contains HCV and HAV RNA, HBV DNA, and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The HEPV_BR384 assay verification was carried out as a 4-plex assay, which simultaneously detects RNA from HAV, HBV, HCV 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 were RNA extracts, 500 bp synthetic DNA constructs (from Twist Biosciences) harboring the regions of interest from the pathogen genes, human RPP30 DNA gene, and human genomic DNA. The results of these experiments are shown in **Figure 1** and indicate that the 4-plex specifically detects the different pathogens in the human genomic DNA matrix.

The limit of detection (LOD) is <10 copies/reaction for all targets, which was determined using serial dilutions (**Figure 2**).

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.

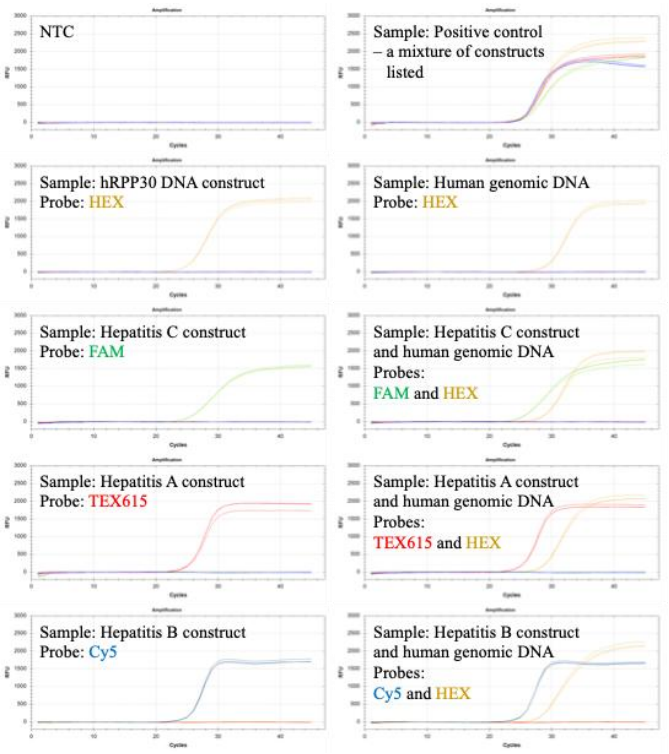

Figure 1: Verification experiments with single and multiple targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

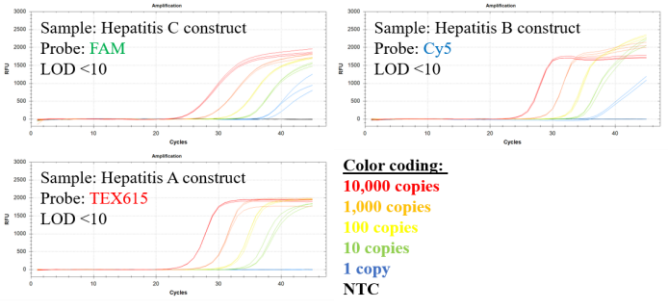

Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

NOTES

[1] FAM™ (Carboxyfluorescein) is a trademark of Life Technologies, Inc

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

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

[4] TEX615™ is a trademark of Thermo Fisher Scientific.

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

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

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

CONTACT US

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

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