PCRassays.com

ASSAY NAME: RPP30RNA (control)

Quantity: 100 x 20µL PCR reactions

1-plex assay: A positive control for human samples to specifically detect spliced RPP30 mRNA in a singleplex or multiplex reactions.

SKU#'s:
PNP-HRNA-BR (Bio-Rad)
PNP-HRNA-QS (QuantStudio)

(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 (BioRad, QuantStudio, MIC). The verification data presented in this document were performed using SKU: PNP-HRNA-BR 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

Tube 1 of this assay contains primers/probe (20X concentrated) targeting spliced human RPP30 mRNA as a RT-qPCR positive control assay for human samples. Note: the primers and probe in this assay differ from those in PNP-HRNA2 from PCRassays.com. The design of this assay makes it specific for mRNA and not for genomic DNA.

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
Human RPP30RNA	HEX	BHQ-1	1, 2

The probes are designed as TaqMan[3] cleavage mechanism using a DNA polymerase with 5'-exonuclease activity (we recommend InhibiTaq Standard RT-qPCR Master Mix).

ASSAY HANDLING AND CONTAMINATION

The RPP30RNA bundle is shipped on ice, and should be stored at -20 °C. The assay 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 specific for human spliced RPP30 mRNA.

Tube 2: (Do NOT add to specimen unknowns) Positive control: Synthetic 500 bp DNA fragment corresponding to spliced RPP30 mRNA.

Tube 3: InhibiTaq Standard 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

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

Component	Volume (µL)
InhibiTaq Standard RT-qPCR enzyme mastermix (2X)	10
RPP30RNA Primer/Probe mix (20X)	1
Sample	2
Water	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 (NOT included) should be used to prepare the PCR reactions.

A PCR protocol was used for verification on a Bio-Rad CFX96™ Real-Time System, 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 15 seconds*
5	Plate Read
6	Go to Step 3, repeat 44× more

*For QuantStudio instruments, we recommend a Step 4 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 > "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.

Sample results below display typical results with a hypothetical Pathogen and using the RPP30RNA assay as the internal control.

Target Pathogen **Fluorphore**[M]	hRPP30 **HEX**™	Recommended Interpretation
–	–	The PCR reaction failed. Please repeat the experiment
–	+	The sample contains the human RPP30 RNA (or spliced-DNA if the spliced DNA control is used). The sample doesn't contain Target RNA.
+	–	The sample contains the **Pathogen** RNA/DNA. The sample may not contain RPP30 mRNA.
+	+	The sample contains the **Pathogen** RNA/DNA, and human RPP30 mRNA.

VERIFICATION EXPERIMENTS

The RPP30RNA assay verification was carried out as a 2-plex assay with PCRassays.com product RSVB (Human respiratory syncytial virus type B), which simultaneously detects RNA from spliced human RPP30 mRNA and RSVB RNA.

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 extracted RSVB (from a human sample from Assurance Scientific Labs) and 100X dilution of human total brain RNA (Takara). The results of these experiments are shown in **Figure 1**.

Conclusion: The data in **Figure 1** indicate that DNAS RPP30RNA kit is compatible with RSVB assay in a 2-plex application to detect RSVB in the matrix of human sample.

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NOTES

[1] HEX™ (Hexachloro-fluorescein) is a trademark of Applera Corp.

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

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

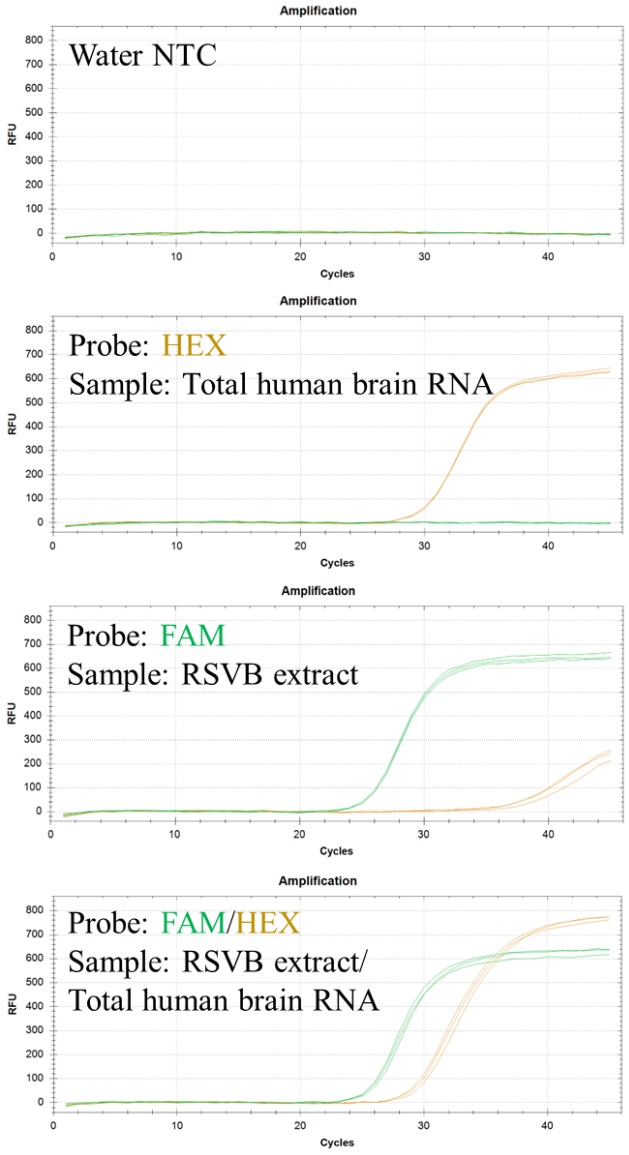

Figure 1: Verification experiments (using a 2-plex reaction of RPP30RNA and RSVB assay from PCRassays.com) with single or double target(s) (given in text boxes for each panel) and human mRNA. Both sets of probes and primers are present in every reaction, but positive signal is only observed for one target at a time, indicating that the amplification is specific. The **HEX** probe detects spliced human RPP30 mRNA. The **FAM** probe detects RSVB. The low HEX signal in the RSVB sample is due to residual human RNA in the RSVB extract from a human sample.

CONTACT US

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

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