PCRassays.com

ASSAY NAME: RPP30DNA (control)

Quantity: 100 x 20µL PCR reactions

1-plex assay: A positive control designed to specifically for human samples to detect intron I of the human RPP30 gene in a singleplex or multiplex reaction.

SKU#'s:
PNP-HDNA-BR (Bio-Rad CFX instrument)
PNP-HDNA-QS (QuantStudio instrument)
PNP-HDNA-MIC (BMS MIC instrument)

(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 pre-validation data presented in this document were performed using SKU: PNP-HDNA-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 targeting human RPP30DNA Intron I (20X concentrated) as a PCR positive control assay for human samples. Because the primers amplify the intron region of the gene, this assay will only detect amplification from genomic DNA and will not detect spliced mRNA. The probes are designed as TaqMan[3] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity (we recommend InhibiTaq Standard qPCR Master Mix).

Tube 2 (optional if ordered) contains a synthetic 500 bp DNA construct containing the amplicon region of the intron I of human RPP30DNA is provided as a positive extraction control. The concentration the DNA construct is approximately 5,000 copies/µL. The Control DNA constructs are for validation purposes only and **Tube 2 should NOT be added to wells for specimen unknowns**.

ASSAY HANDLING AND CONTAMINATION

The RPP30DNA assay is shipped at ambient temperature, 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.

Note: molecular biology grade water should be used to prepare the PCR reactions (NOT included in this assay).

Assay contents:

Tube 1: 20X Primer/Probe mix specific for intron I of the human RPP30 gene.

Tube 2: (optional if ordered) 5000 copies/µl Positive control of synthetic 500 bp DNA fragment of RPP30DNA.

Tube 3: (optional if ordered) 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.

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
Human RPP30DNA	HEX	BHQ-1	1, 2

EXPERIMENTAL

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

Component	Volume (µL)
InhibiTaq Standard qPCR enzyme mastermix (2X)	10
RPP30DNA Primer/Probe mix (20X)	1
(optional) Pathogen Primer/Probe mix (20X)	1
Sample	2
Water	6

Notes: To improve assay sensitivity, up to 8 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 (i.e., the "sample").

A PCR protocol was used 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 3, repeat 44xmore

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 > "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 with a hypothetical Pathogen and using RPP30DNA as the internal control.

Target Pathogen FluorphoreM	hRPP30 HEX™	Recommended Interpretation
–	–	The PCR reaction failed. Please repeat the experiment
–	+	The sample doesn't contain the target DNA.
+	–	The sample contains the Pathogen DNA. The sample may not contain RPP30DNA DNA.
+	+	The sample contains the Pathogen DNA, and RPP30DNA DNA.

VERIFICATION EXPERIMENTS

The RPP30DNA assay verification was carried out as a duplexed assay with PCRassays.com product AdenoB1, which simultaneously detects DNA from intron I of the human RPP30 gene and the human adenovirus B1 DNA.

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 $1x10^4$ copies/reaction of synthetic 500 bp synthetic DNA constructs (from Twist Biosciences) harboring the regions of interest from the RPP30DNA gene and human Adenovirus B1 genome. The results of these experiments are shown in **Figure 1**.

Conclusion: The data in **Figure 1** indicate that the RPP30DNA assay can detect RPP30DNA genomic DNA and serve as an internal control assay in multiplexed PCR reactions.

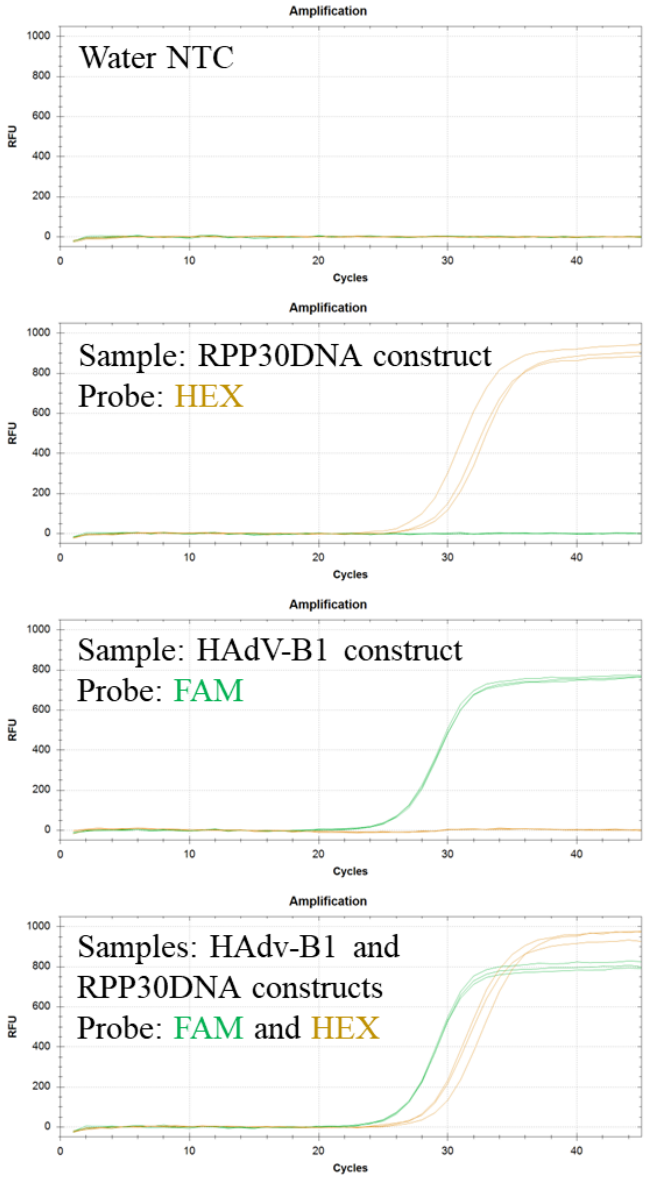

Figure 1: Verification experiments (in a 2-plex reaction of RPP30DNA and HAdv-B1 assay from PCRassays.com) with single or double target(s) (given in text boxes for each panel). Both sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific. The **HEX** probe detects RPP30DNA construct DNA. The **FAM** probe detects human Adenovirus B1 construct DNA.

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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.

CONTACT US

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

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