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

SKU#'s: PNP-HDNA-BR

(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 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

The primers and probes in the RPP30DNA assay are provided in Tube 1 as a 20X concentrated working solution that detects the human RPP30 intronic region.

Table of Dyes used in this assay:

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

The probes are designed as TaqMan[3] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'- exonuclease activity.

ASSAY HANDLING AND CONTAMINATION

The RPP30DNA 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 intron I of the human RPP30 gene.

Tube 2: Positive control of synthetic 500 bp DNA fragment of RPP30DNA.

Tube 3: 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.

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
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 (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

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.

hRPP30 HEX™	Recommended Interpretation
–	The sample does not contain hRPP30 DNA. It is also possible that the PCR reaction failed, so a repeat is advised.
+	The sample contains hRPP30 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.

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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.

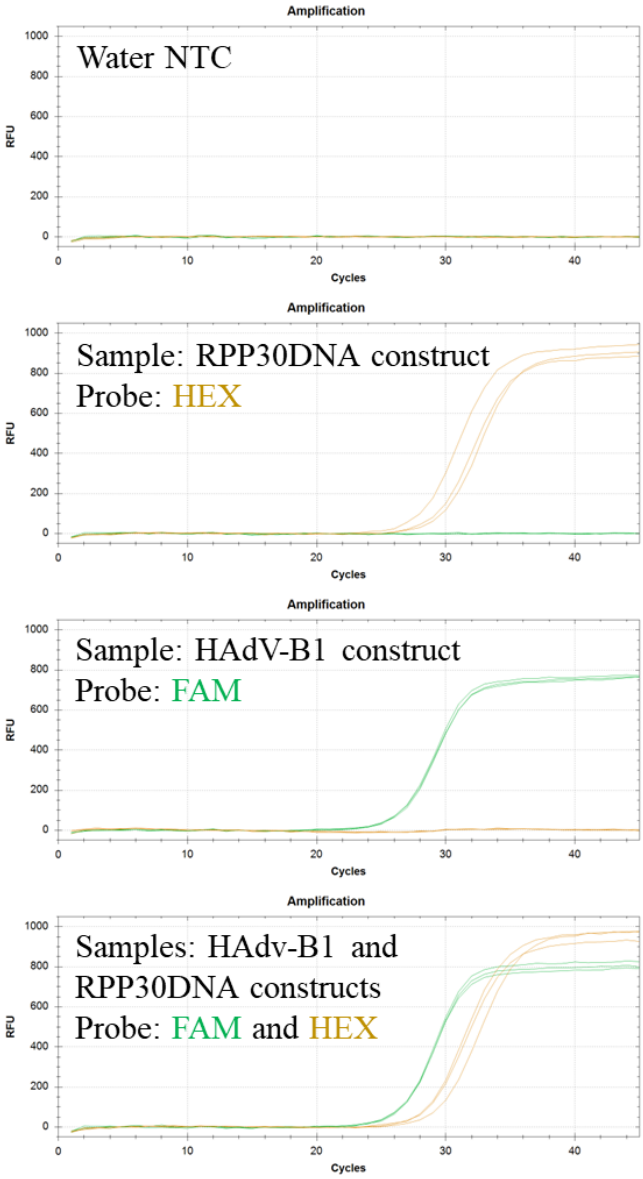

Figure 1: Verification experiments (in a 2-plex reaction of RPP30DNA and HAdv-B1 assay from PCRassays.com) with single or double target(s) (given in text boxes for each panel). Both sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present,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

CONTACT US

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

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