human RPP30 mRNA kit (100 reactions): A positive control designed to specifically detect spliced hRPP30 mRNA in singleplex or multiplex reactions.
(RUO). Research Use Only. Not for use in Diagnostic Procedures.

INTRODUCTION/ASSAY DESIGN

The DNA Software positive control kit for detection of human RPP30 mRNA is intended to verify the validity of a real-time reverse transcriptase polymerase chain reaction (RT-PCR) on a sample of human origin. This kit is for research use only and should not be used for diagnostic procedures.

There have been a number of RPP30 positive controls published in the literature.[1,2] The RPP30 assay published by the CDC[1] works for detection of human genomic DNA and RNA, but does not discriminate between DNA and RNA, thus is not an appropriate control for a reverse transcription reaction.[2] In addition, DNA Software has found that the Forward Primer from the CDC RPP30 assay is prone to formation of homodimers and also cross-reacts with many amplicons in multiplexed reactions due to the GC-rich content of the 3'-end of the CDC FP primer. A revised assay was published by Timo Briet's group,[2] which redesigned the RP so that it is specific for spiced mRNA by binding to exon 2 and partially straddling the exon-exon boundary. This results in selective amplification of only RNA, which makes it an appropriate control for RT-PCR reactions. However, the revised assay utilizes the CDC FP, and thus also forms homodimers and cross-reacts with other amplicons in multiplexed reactions. The assay from DNA Software (*i.e.*, this kit) is designed so that FP binds in exon 1, the probe straddles the exon-exon boundary, and the RP binds to exon 2. Genomic DNA is not amplified because the intron is 2801 nts. long and even if a little amplification did occur, the probe will not bind to such an amplicon from human genomic DNA and thus no signal from genomic DNA is expected. Several experiments performed at DNAS (**Figures 1 and 2,** and additional data not shown) indicate that the DNAS RPP30 mRNA kit is specific to spliced human RPP30 mRNA, no cross reactivity was observed with human genomic DNA, and no cross-reactivity of the assay was observed in multiplexed reactions.

CONTENTS

A mix of primers/probe targeting human RPP30 mRNA is provided in a tube (a 20X concentrated working solution). The fluorophore of the probe is HEX™ (Hexachloro-fluorescein, a trademark of Life Technologies, Inc), and the quencher is BHQ-1™ (Black Hole Quencher, a trademark of Biosearch Technologies, Inc.).

Note: molecular biology grade water should be used to prepare the PCR reactions, which is NOT included in this kit.

Kit contents:
Tube 1: 20X primers/probe specific for mature (*i.e.,* spliced) human RPP30 mRNA.

An RT-PCR protocol was used in-house for pre-validation on a Bio-Rad CFX96™ Real-Time System, with the following program:

Step	Thermocycling Protocol:
1	Incubate @ 50 °C for 5 minutes
2	Incubate @ 94 °C for 3 minutes
3	Incubate @ 94 °C for 5 seconds
4	Incubate @ 63 °C for 30 seconds
5	Plate Read
6	Go to Step 3, repeat 44x more
7	(optional) Incubate @63 °C for 3 minutes

KIT HANDLING AND CONTAMINATION

The DNA Software RNA human RPP30 is shipped at room temperature but should be stored at -15 °C of lower for long term storage. The kit should be kept on ice once thawed.

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

EXPERIMENTAL

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

Component	Volume (µL)
TaqMan mastermix	10
RT enzyme	0.5
Target(s) primers/probe mix	1
RPP30 mRNA primers/probe mix	1
Sample	2
Water	5.5

Note: The volume of water should be adjusted accordingly if the user's reaction preparation is different from the recommended preparation method.

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 is considered "positive" or "+" in the Table below.

Target RNA (Fluorophore)	**RPP30 (HEX™)**	Interpretation and recommendation
–	–	The PCR reaction failed. Please repeat the experiment
–	+	The sample doesn't contain the target RNA.
+	–	The sample contains the target RNA. The sample may not contain human RPP30 mRNA.
+	+	The sample contains the target RNA, and human RPP30 mRNA.

PRE-VALIDATION

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 included water (NTC), human genomic DNA (3100 copies, Clontech), human brain RNA (1500 copies, Roche), and a mixture of human genomic DNA and human brain RNA (3100 and 1500 copies, respectively). The reverse transcriptase and TaqMan mastermix from Empirical Bioscience (items: RTqPCR-Kit-200) were employed. The results of these experiments are shown in **Figure 1** below:

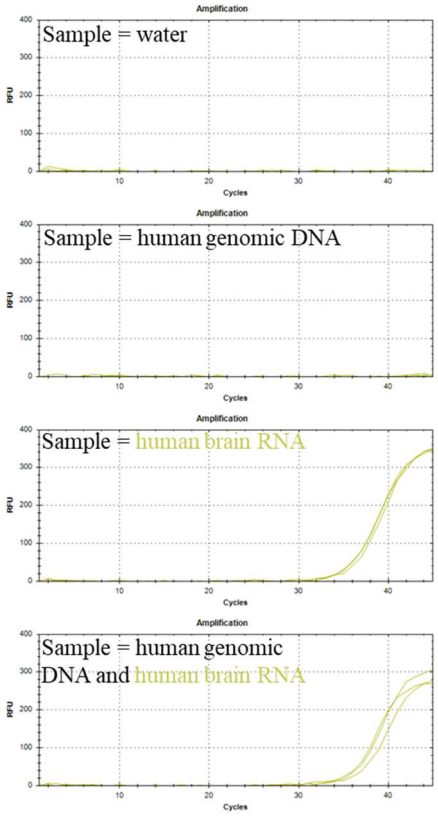

Figure 1: Validation experiments with water, human genomic DNA, human brain RNA, and a mix of human genomic DNA and human brain RNA. The **HEX** probe detects spliced human RPP30 mRNA (in human brain RNA sample).

The human RPP30 mRNA positive control was included in pre-validation of DNA software SARS-CoV-2 kits. For example, when the primers and probe of S1P (Omicron BA.1 specific kit from DNA Software) in combination with the primers and probe of human RPP30 mRNA positive control were used to test one of the three standard RNAs (1×10^5 copies/reaction), SARS-CoV-2 Delta variant (Twist® Standard RNA #18), Omicron BA.1 variant (Twist® Standard RNA #48), and Omicron BA.2 variant (Twist® Standard RNA #50), mixed with human genomic DNA (Clontech, 3100 copies) / human brain RNA (Roche, 1500 copies), **HEX** signal was observed in all samples and FAM signal from S1P kit was observed only in Omicron BA.1 samples. The results of these experiments are shown in **Figure** 2 below:

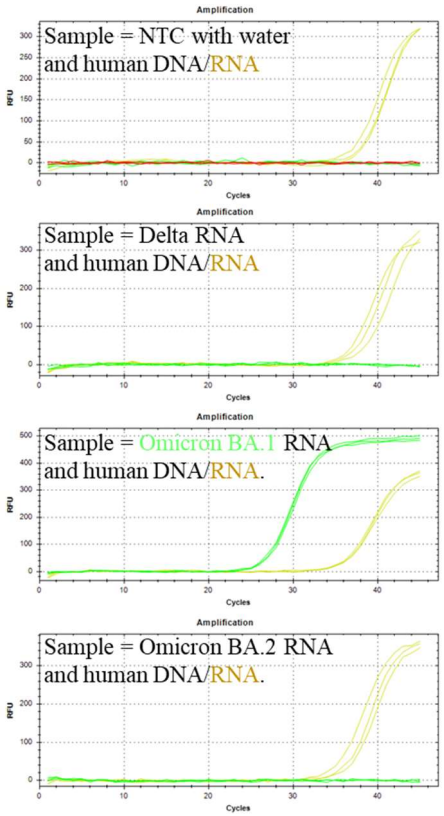

Figure 2: Application of human RPP30 mRNA positive control (**HEX** signal) in a biplex reaction with DNA software S1P kit (FAM signal).

Conclusion: The data in **Figure 1** indicate that the RNA human RPP30 kit specifically detects spliced human RPP30 mRNA but does not detect RPP30 gene in human genomic DNA. The data in **Figure 2** indicate that the DNAS human RPP30 RNA positive control primers and probe are compatible with other RT-PCR kits for multiplexed reactions.

CONTACT US

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LITERATURE REFERENCES

1. Research Use Only 2019-Novel Coronavirus (2019-nCoV) Real-time RT-PCR Primers and Probes. Centers for Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html
2. Dekker, R.J., Ensink, W.A., Breit, T.M., *et al*. Overhauling a faulty control in the CDC-recommended SARS-CoV-2 RT-PCR test panel. bioRxiv (2020) doi: https://doi.org/10.1101/2020.06.12.147819