PCRassays.com

ASSAY NAME: SARS-CoV-2 N1P_D_QS

Quantity: 100 x 20µL PCR reactions

2-plex assay: SARS-CoV-2 N1P and human RPP30 DNA

Detects: Omicron variants: BA.1, BA.2, BA.4 and BA.5 through JN.1, KP.2, KP.2.3, KP.3, KP.3.1.1, LB.1, LB.1.3, LF.7, LP.8.1, MC.1, XEC (checked 2-12-2025)
Targets: N gene: Δ31-33

SKU#'s: BUN-N1P-D-QS-100

(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 verification data presented in this PIS document were performed using BUN-N1P-D-QS-100 on a QuantStudio 7 instrument. The performance of the other SKUs on their corresponding instrument should be similar. Contact PCRassays.com if need to use a different qPCR instrument.

CONTENTS

The primers and probes in the SARS-CoV-2 N1P-D-QS kit are provided in Tube 1 as a 5X concentrated working solution. The same mix also contains primers/probe targeting human RPP30DNA as a positive control assay for human samples.

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
RPP30-DNA control	TAMRA	BHQ-2	3, 4
SARS-CoV-2 N1P	FAM	BHQ-1	1, 2

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

ASSAY HANDLING

The SARS-CoV-2 N1P-D-QS 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 SARS-CoV-2 N1P and RPP30-DNA.

Tube 2: (Do NOT add to specimen unknowns) Positive control: Synthetic 500 bp DNA fragments of SARS-CoV-2 N1P region and hRPP30DNA.

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

Perform nucleic acid extraction/purification (recommended).

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

Component	Volume (µL)
InhibiTaq Standard RT-qPCR enzyme mastermix (2X)	10
Primer/Probe mix (5X)	4
Sample (or positive control from tube 2)	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.

An RT-PCR protocol was used at PCRassays.com for verification on a QuantStudio 7 instrument, with the following program.

Step	Thermocycling Protocol:
1	Incubate @ 50 °C for 10 minutes
2	Incubate @ 95 °C for 2 minutes
3	Incubate @ 95 °C for 5 seconds
4	Incubate @ 55 °C for 22 seconds
5	Plate Read
6	Go to Step 3, repeat 44× 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 cannot compute Cq). Fluorescence channels with a Cq < 37 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 QuantStudio 7 with 96 well plate with 100 µL wells and 20 µL reaction volume, the average RFU was approximately 1,000,000 and we used a threshold of 200,000 for calling positives or "+" in the Table below.

SARS CoV2 N1P FAM™	hRPP30 TAMRA™	Recommended Interpretation
–	–	The PCR reaction failed. Please repeat the experiment.
–	+	The sample does not contain viral RNA of interest. The sample contains human RPP30 DNA.
+	–	The sample contains SARS CoV2 N1P RNA. The sample may not contain human RPP30 DNA.
+	+	The sample contains SARS CoV2 N1P RNA and human RPP30 DNA.

VERIFICATION EXPERIMENTS

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 1×10^5 copies/reaction synthetic viral RNA obtained from Twist Biosciences: SARS-CoV-2 Omicron BA.2 variant (Twist® Standard RNA #50). The samples also contained human genomic DNA (3100 copies) from Clontech. The presence of the human genomic DNA in the reaction appears to have no effect on the amplification of SARS-CoV-2 RNA with this kit. The results of these experiments are shown in **Figure 1**.

Limit of detection (LOD) was estimated by performing serial dilution experiments in triplicate (**Figure 2**). The results show a limit of detection (LOD) <10 copies/reaction for both templates.

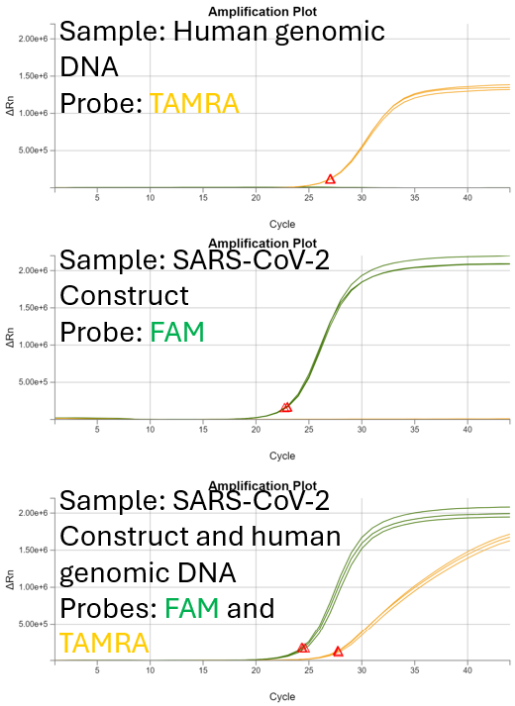

Figure 1: Verification experiments with single or double target(s) (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific. The **FAM** probe detects SARS-CoV-2 Omicron variants. The **TAMRA** probe detects human RPP30 DNA.

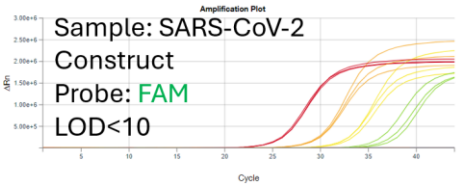

Figure 2: Serial dilution experiments show LOD <10 molecules for each target.

Conclusion: The data in **Figure 1** indicates that the SARS-CoV-2 N1P primers and probe are compatible with the human RPP30 DNA positive control primers and probe.

CONTACT US

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

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46701 Commerce Center Dr, Plymouth, MI 48170

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NOTES

[1] FAM™ (Carboxyfluorescein), a trademark of Life Technologies Corporation.

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

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

[4] TAMRA™ (Carboxyltetramethylrhodamine) is a trademark of Applera Corp.

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