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siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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ASSAY NAME: SARS CoV2 N1PN2P (all variants)

Quantity: 100 x 20µL PCR reactions

3-plex assay: SARS CoV2 N1PN2P (all variants) and human RPP30 mRNA

Detects: N1P detects Omicron variants: BA.1, BA.2, BA.4 and BA.5 through JN.1. N2P detects Wuhan strain or later alpha, beta, gamma, or delta VOCs. Human RPP30 mRNA (positive control).

Targets: N gene: Wild type and Δ31-33 strains

SKU#'s:
PNP-COVN-R-BR (Bio-Rad with RPP30 assay)
PNP-COVN-N-BR (Bio-Rad without control assay)
PNP-COVN-R-QS (QuantStudio with RPP30 assay)
PNP-COVN-N-QS (QuantStudio without control assay)
PNP-COVN-R-MIC (BMS MIC with RPP30 assay)
PNP-COVN-N-MIC (BMS MIC without control assay)

(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 document were performed using PNP-COVN-D-BR-100 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 SARS CoV2 N1PN2P assay are provided in Tube 1 as a 20X concentrated working solution. The same mix also contains primers/probe targeting the RPP30 mRNA splice junction as a qPCR positive control assay for human samples. The probes are designed as TaqMan[6] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity (we recommend InhibiTaq Standard qPCR Master Mix).

Table of Dyes used in this kit:

Pathogen/Target	Dyes	Quencher	Refs.
RPP30-RNA control	HEX	BHQ-1	3, 4
SARS CoV2 N1P	FAM	BHQ-2	1, 2
SARS CoV2 N2P	CalFluorRed 610	BHQ-2	5, 2

Tube 2 "double positive control" contains a mixture of synthetic 500 bp DNA constructs containing the amplicon regions of all targets and human RPP30 is provided as a positive extraction control. The concentration of each DNA construct is approximately 5,000 copies/µL. The DNA constructs are for validation purposes only and **Tube 2 should NOT be added to wells for specimen unknowns**.

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

Assay contents:

Tube 1: 20X Primer/Probe mix for SARS CoV2 N1PN2P. If you order SKU#: PNP-COVN-R, then primers and probes for detecting human RPP30 mRNA are also included. Alternative control for PMMoV for wastewater samples is also available (inquire).

Tube 2: (optional if ordered) 5000 copies/µl Positive controls of synthetic 500 bp DNA fragments of SARS CoV2 N1PN2P and hRPP30.

Tube 3: (optional if ordered) Spike-in control. 1.0E6 copies/uL of synthetic 500 BP regions of SARS CoV2 N1PN2P and DNA corresponding to human RPP30 mRNA.

Tube 4: (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.

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KIT HANDLING AND CONTAMINATION

The SARS CoV2 N1PN2P assay is shipped at ambient temperature, and should be stored at -20 °C. The kit 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.

EXPERIMENTAL

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

Component	Volume (µL)
InhibiTaq Standard qPCR enzyme mastermix (2X)	10
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").

An RT-PCR protocol was used at PCRassays.com for verification 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 @ 60 °C for 30 seconds
5	Plate Read
6	Go to Step 3, repeat 44x more
7	(optional) Incubate @60 °C for 3 minutes

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 < 37 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.

Omicron (FAM™)	non-Omicron VOC (CalFluorRed610™)	RPP30 (HEX ™)	Recommended Interpretation
–	–	–	The PCR reaction failed. Please repeat the experiment.
–	–	+	The sample doesn't contain SARS-CoV-2 VOC RNA.
+	–	–	The sample contains SARS-CoV-2 Omicron variant RNA. The sample may not contain spliced human RPP30 mRNA.
+	–	+	The sample contains SARS-CoV-2 Omicron variant RNA and spliced human RPP30 mRNA.
–	+	–	The sample contains SARS-CoV-2 non-Omicron VOC RNA. The sample may not contain spliced human RPP30 mRNA.
–	+	+	The sample contains SARS-CoV-2 non-Omicron VOC RNA and spliced human RPP30 mRNA.
+	+	–	The sample contains SARS-CoV-2 non-Omicron VOC RNA and Omicron variant RNA. The sample may not contain spliced human RPP30 mRNA
+	+	+	The sample contains SARS-CoV-2 non-Omicron VOC RNA and Omicron variant RNA and spliced human RPP30 mRNA.

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 verification experiments contained 1×10^5 copies/reaction synthetic viral RNA obtained from Twist Biosciences as follows:

SARS-CoV-2 Delta variant (Twist® Standard RNA #18)
SARS-CoV-2 Omicron BA.1 variant (Twist® Standard RNA #48)
SARS-CoV-2 Omicron BA.2 variant (Twist® Standard RNA #50)
The samples also contained human brain RNA (1500 copies) from Roche and human genomic DNA (3100 copies) from Clontech. The RPP30 control primers and probe specifically reverse transcribe and amplify the human RPP30 mRNA and not genomic DNA. The presence of the human genomic DNA in the reaction appears to have no effect on the amplification of SARS-CoV-2 RNA (data not shown). These experimental results are shown in **Figure 1**.

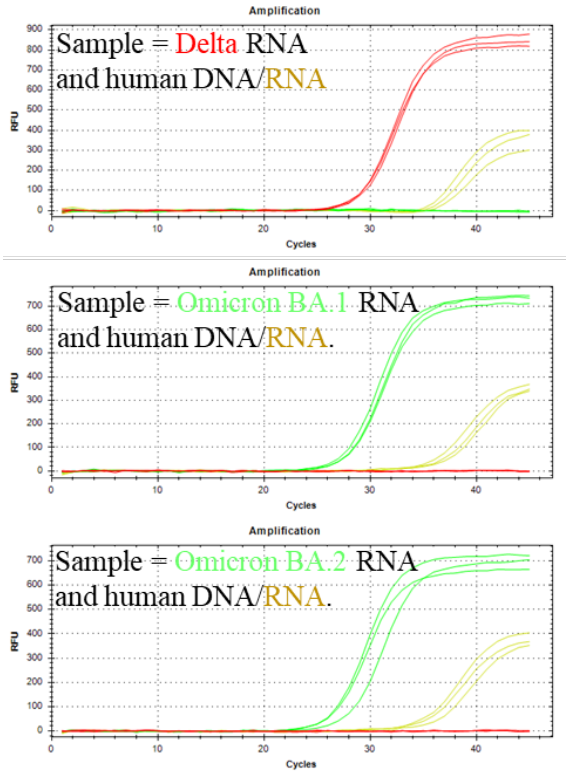

Figure 1: Validation experiments with single targets (given in text boxes for each panel) and human mRNA. All three probes and primers are present in every reaction, but positive signal is only observed for one target at a time, indicating that the amplification is specific.

Limit of detection (LOD) was estimated by performing serial dilution experiments in triplicate (**Figure 2**, next page). For dilution series only one SARS CoV-2 template RNA was added (*i.e.,* Omicron BA.1, or Omicron BA.2). The results show a limit of detection (LOD) <20 copies/reaction for both templates.

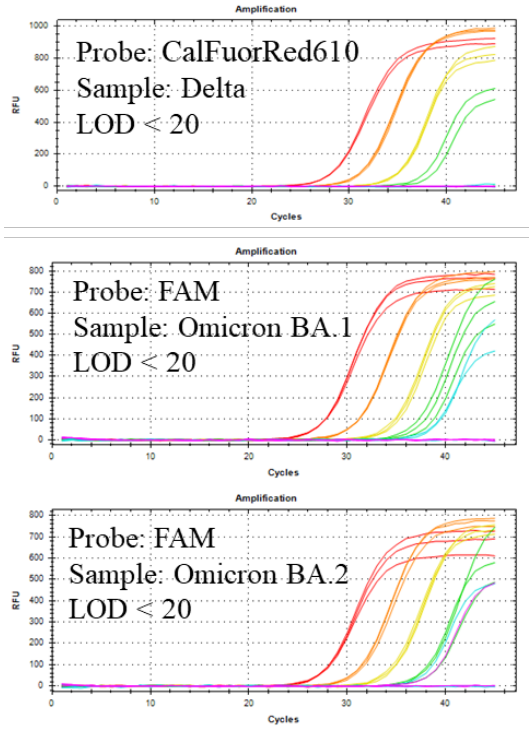

Figure 2: Serial dilution experiments show LOD <20 molecules for each target. For the bottom panel (BA.2), 1 out of the 3 NTC reactions showed an amplification with Cq=37.4 (the other 2 NTC reactions were flat). There may have been a single molecule contamination in that reaction since we have not observed that in any of the other many NTC reactions run for this assay.

Conclusion: The data in **Figure 1** indicate that the N1P-N2P-RPP30 assay specifically detects Delta, Omicron BA.1 and Omicron BA.2 variants of SARS CoV-2. Based on sequence analysis, it can be inferred that the N1P-N2P-RPP30 assay will also detect in the CalFluorRed Channel the reference SARS CoV-2 (i.e. Wuhan strain) and other earlier VOCs such as alpha, beta, and gamma (data not shown).

CONTACT US

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

Address: Michigan Life Science and Innovation Center,
46701 Commerce Center Dr, Plymouth, MI 48170

Phone: (734) 222-9080

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NOTES

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

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

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

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

[5] CalFluorRed610™ is a trademark of Thermo Fisher Scientific.

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