Assay: N1P-N2P-RPP30 (100 reactions):
SARS CoV-2 Variant of Concern
with RPP30 control,
N-gene: Δ31-33
PCR mastermix included
(RUO). Research Use Only. Not for use in Diagnostic Procedures.
Cat #: N1PN2P-TCE-0003

Kit contents:
Tube 1: 20X primers/probe specific for SARS CoV-2 VOCs.
Tube 2: 20X primers/probe specific for spliced human RPP30 mRNA.
Tube 3: 2X TaqMan mastermix.
Tube 4: 40X RT enzyme.
(Tubes 3 and 4 are from Empirical Biosciences).

INTRODUCTION

The DNA Software assay N1P-N2P-RPP30 is a real-time reverse transcriptase polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 Omicron variants (BA.1 and BA.2) with FAM™ (Carboxyfluorescein, a trademark of Life Technologies, Inc) and VOCs before Omicron (*i.e.*, original Wuhan strain or later alpha, beta, gamma, or delta VOCs) with CalFluorRed610™ (a trademark of Biosearch Technologies, Inc). This kit is for research use only and should not be used for diagnostic procedures.

This kit is pre-validated with three standard synthetic RNA genomes from Twist Biosciences: SARS-CoV-2 Delta variant (Twist® Standard RNA #18), Omicron variant BA.1 (Twist® Standard RNA #48) and Omicron variant BA.2 (Twist® Standard RNA #50). The limit of detection (LOD) is below 20 copies/reaction for the three variants. The FAM™ probe is specific to SARS-CoV-2 Omicron variants (both BA.1 and BA.2) and no cross-reactivity was observed with a standard synthetic RNA of SARS-CoV-2 Delta variant (Twist® Standard RNA #18). The CalFluorRed610™ probe is specific to SARS-CoV-2 Delta variant (and the variants prior to Delta by sequence alignment) and no cross-reactivity was observed with the standard synthetic RNAs of SARS-CoV-2 Omicron variants (Twist® Standard RNA #48 and #50).

CONTENTS

A mix of primers/probe targeting the RNA region coding for N protein (Δ31-33) in SARS-CoV-2 genome is provided in a tube as a 20X concentrated working solution. The fluorophore of the probe for Omicron variants BA.1 and BA.2 is FAM™ and the quencher is BHQ-1™ (Black Hole Quencher, a trademark of Biosearch Technologies, Inc.). The fluorophore of the probe for Delta and earlier VOCs is CalFluorRed610™ and the quencher is BHQ-2™ (Black Hole Quencher, a trademark of Biosearch Technologies, Inc.). A mix of primers/probe targeting spliced human RPP30 mRNA is also provided in a tube (a 20X concentrated working solution) as a RT-PCR positive control for human samples. The fluorophore of the probe is HEX™ (Hexachloro-fluorescein, a trademark of Life Technologies, Inc), and the quencher is BHQ-1™. An alternative RNA positive control of PMMoV is also available for wastewater samples as a 20X concentrated working solution with HEX™ fluorophore. Other positive control(s) should be used in place of the human RPP30 mRNA or PMMoV primers/probe if the samples are originated from other sources. Users are responsible to provide such alternative control(s).

The QuantiTASE PLUS One-Step RT-qPCR Kit from Empirical Bioscience (items: SKU-KIT-200) are included in this kit. This Empirical kit provided reproducible and reliable results in prevalidation experiments and is recommended for applications with the N1P-N2P-RPP30 kit. See EXPERIMENTAL for more details.

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

KIT HANDLING AND CONTAMINATION

The DNA Software Assay N1P-N2P-RPP30 is shipped with ice packs, and should be stored at -30 to -15°C. 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 (2X)	10
RT enzyme (40X)	0.5
N1P-N2P primers/probe mix (20X)	1
RPP30 mRNA primers/probe mix (20X)	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.

An RT-PCR protocol was used at DNA Software, Inc. 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

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.

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.

PRE-VALIDATION DATA

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 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 (See DNAS Product insert about RPP30 RNA control for more information). 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). The results of these experiments are shown in **Figure 1** below:

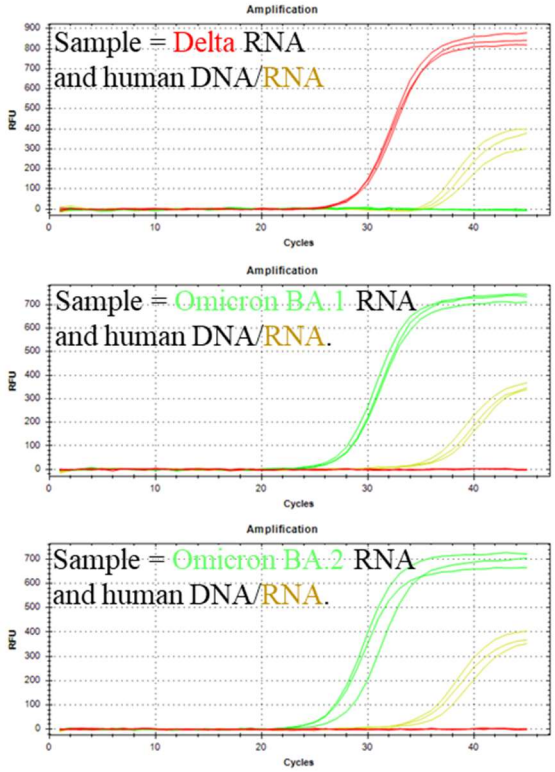

Figure 1: Validation experiments with single targets (given in text boxes for each panel) and human mRNA. All three probes and primers are present in every reaction, but positive signal is only observed for one target at a time, indicating that the amplification is specific. The **CalFluorRed610** probe detects the Delta variant (Twist #18). The **FAM** probe detects Omicron BA.1 and BA.2. The **HEX** probe detects spliced human RPP30 mRNA.

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).

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

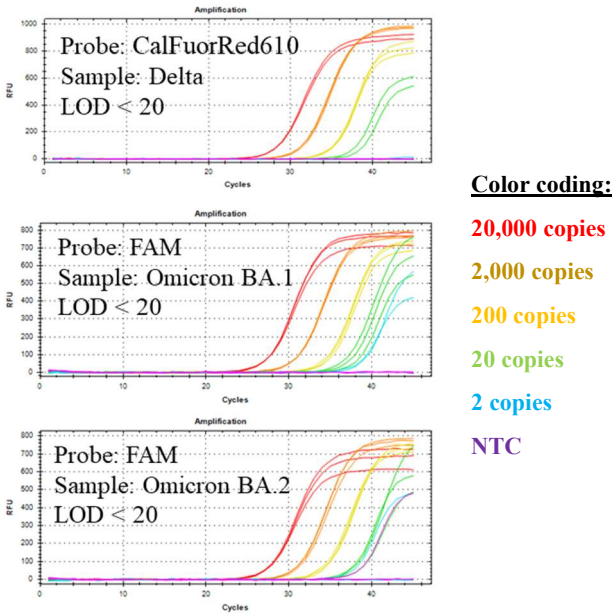

Figure 2: Serial dilution experiments show LOD <20 molecules for each target. For the bottom panel (BA.2), 1 out of the 3 NTC reactions showed an amplification with Cq=37.4 (the other 2 NTC reactions were flat). We think there may have been a single molecule contamination in that reaction since we have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

CONTACT US

For further assistance, please contact DNA Software using the link: https://www.dnasoftware.com/contact/

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