



**Assay: S2P-RPP30 (100 reactions):  
SARS CoV-2 Omicron variant BA.2  
with RPP30 control,  
S-gene: Δ25-27  
(RUO). Research Use Only. Not for use in  
Diagnostic Procedures.  
SKU: PNP-S2P-R-100**

**Kit contents:**

Tube 1: 20X Primers/Probe specific for Omicron BA.2.  
Tube 2: 20X primers/probe specific for spliced human RPP30 mRNA.

**INTRODUCTION**

The DNA Software assay S2P-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 variant BA.2 (S-gene: Δ25-27). This kit is for research use only and should not be used for diagnostic procedures.

The performance of this kit was tested using 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 100 copies/reaction. This kit is specific to SARS-CoV-2 Omicron variant BA.2 and no cross reactivity was observed with standard synthetic RNAs of SARS-CoV-2 Delta variant (Twist® Standard RNA #18) and SARS-CoV-2 Omicron variant BA.1 (Twist® Standard RNA #48).

**CONTENTS**

A mix of primers/probe targeting the RNA region coding for S protein (Δ25-27) in SARS-CoV-2 Omicron variant BA.2 genome is provided in a tube as a 20X concentrated working solution. The fluorophore of the probe is FAM™ (Carboxyfluorescein, a trademark of Life Technologies, Inc) and the quencher is BHQ-1™ (Black Hole Quencher, a trademark of Biosearch Technologies, Inc.) A tube with a mix of primers/probe targeting spliced human RPP30 mRNA is also provided (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).

**KIT HANDLING AND CONTAMINATION**

The DNA Software Assay S2P-RPP30 is shipped at ambient temperature, 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)
TaqPath™ mastermix (4X)	5
S1P primers/probe mix (20X)	1
RPP30 mRNA primers/probe mix (20X)	1
Sample	2
Water	11

**Note: The composition of this reaction is calculated based on the user manual of TaqPath™ 1-step RT-qPCR Master Mix, Applied Biosystems™.**

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 @ 60 °C for 30 seconds
5	Plate Read
6	Go to Step 3, repeat 44x more
7	(optional) Incubate @60 °C for 3 minutes

## RESULT INTERPRETATION

After running the qPCR reaction, perform a regression analysis on the data to determine the quantification cycle, C<sub>q</sub>. (C<sub>q</sub> is preferred over C<sub>t</sub>). Each fluorescence channel with a C<sub>q</sub> < 38 cycles and final RFU >200 is considered “positive” or “+” in the Table below.

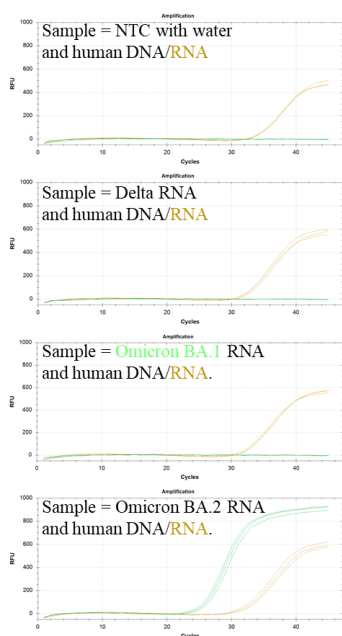
Omicron BA.2 (FAM™)	RPP30 (HEX™)	Recommended Interpretation
–	–	The PCR reaction failed. Please repeat the experiment
–	+	The sample doesn't contain SARS-CoV-2 Omicron variant BA.2 RNA.
+	–	The sample contains SARS-CoV-2 Omicron variant BA.2 RNA. The sample may not contain spliced human RPP30 mRNA.
+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

## PRE-VALIDATION EXPERIMENTS

Experiments were performed at DNA Software, Inc. 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<sup>5</sup> 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)

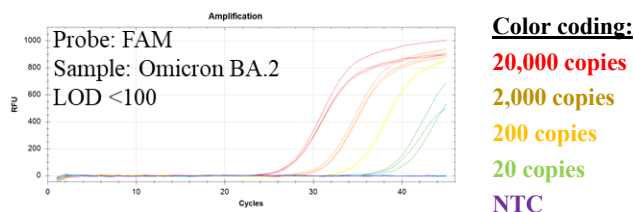
The samples also contained human brain RNA (1500 copies) from Roche and human genomic DNA (3100 copies) from Clontech. The RPP30 control primers and probe specifically reverse transcribe and amplify the spliced human RPP30 mRNA and not genomic DNA (See DNAS Product insert about RPP30 mRNA control for 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. Both S2P and human RPP30 mRNA 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 **FAM** probe detects Omicron BA.2. The **HEX** probe detects spliced human RPP30 mRNA.

**Conclusion:** The data in **Figure 1** indicate that the S2P assay specifically detects Omicron BA.2 but does not detect Omicron BA.1 or Delta variants. Based on sequence analysis, it can be inferred that the S2P assay will also not detect the reference SARS CoV-2 (*i.e.*, Wuhan strain) or other earlier VOCs such as alpha, beta, gamma, or delta.

Limit of detection (LOD) was estimated by performing serial dilution experiments in triplicate (**Figure 2**). For dilution series only the SARS CoV-2 Omicron variant BA.2 template RNA was added. The results show a limit of detection (LOD) <100 copies/reaction.



**Figure 2:** Serial dilution experiments show LOD <100 molecules for SARS-CoV-2 Omicron variant BA.2.

## CONTACT US

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

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

Phone: (734) 222-9080