Assay: S1F-RPP30 (100 reactions):
SARS CoV-2 Omicron variant BA.1
with RPP30 control,
S-gene: Δ211, L212I, ins214EPE
PCR mastermix included
(RUO). Research Use Only. Not for use in Diagnostic Procedures.

Cat #: S1F-TCE-0001

Kit contents:
Tube 1: 20X Primers/Probe specific for Omicron BA.1.
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 S1F-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.1 (S-gene: Δ211, L212I, ins214EPE). 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. The assay was also tested by Rapid Bio, Inc. using 80 clinical specimens (saliva swab without RNA extraction). This kit is specific to SARS-CoV-2 Omicron variant BA.1 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.2 (Twist® Standard RNA #50).

CONTENTS

A mix of primers/probe targeting the RNA region coding for S protein (Δ211, L212I, ins214EPE) in SARS-CoV-2 Omicron variant BA.1 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 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 pre-validation experiments and is recommended for applications with the S1F-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 S1F-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
S1F 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 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

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 BA.1 (FAM™)	RPP30 (HEX™)	Recommended Interpretation
–	–	The PCR reaction failed. Please repeat the experiment
–	+	The sample doesn't contain SARS-CoV-2 Omicron variant BA.1 RNA.
+	–	The sample contains SARS-CoV-2 Omicron variant BA.1 RNA. The sample may not contain spliced human RPP30 mRNA.
+	+	The sample contains SARS-CoV-2 Omicron variant BA.1 RNA, and spliced 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 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 spliced human RPP30 mRNA and not genomic DNA (See DNAS Product insert about human 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:

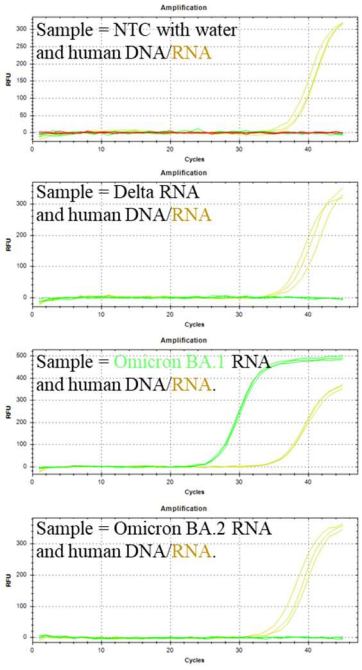
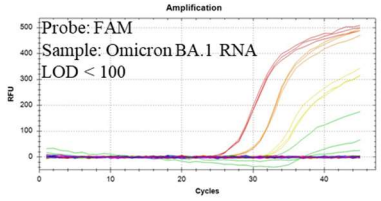


Figure 1: Validation experiments with single targets (given in text boxes for each panel) and human mRNA. Both S1F and human RPP30 mRNA probes and primers are present in every reaction, but positive signal is only observed for one target at a time, indicating that the amplification is specific. The **FAM** probe detects Omicron BA.1. The **HEX** probe detects spliced human RPP30 mRNA.

Conclusion: The data in **Figure 1** indicate that the S1F assay specifically detects Omicron BA.1 but does not detect Omicron BA.2 or Delta variants. Based on sequence analysis, it can be inferred that the S1F 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 one SARS CoV-2 Omicron variant BA.1 template RNA was added. The results show a limit of detection (LOD) <100 copies/reaction.



Color coding:
20,000 copies
2,000 copies
200 copies
20 copies
NTC

Figure 2: Serial dilution experiments show LOD < 100 molecules for SARS-CoV-2 Omicron variant BA.1.

CONTACT US

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