Assay: L452R-Delta (100 reactions):
SARS CoV-2 Delta variant
S-gene: L452R
(RUO). Research Use Only. Not for use in Diagnostic Procedures.
Cat #: L452RDelta-T-0014

Kit contents:
Tube 1: 20X Primers/Probe specific for Delta.

INTRODUCTION

The DNA Software assay L452R-Delta is a real-time reverse transcriptase polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 Delta variant (S-gene: L452R). This kit is for research use only and should not be used for diagnostic procedures.

The performance of this kit was tested using two standard synthetic RNA genomes from Twist Biosciences: SARS-CoV-2 wild type (Twist® Standard RNA #6) and Delta variant (Twist® Standard RNA #18). The limit of detection (LOD) is estimated to be below 100 copies/reaction. This kit is specific to SARS-CoV-2 Delta variant and no cross reactivity was observed with standard synthetic RNAs of SARS-CoV-2 wild type (Twist® Standard RNA #6). Based upon both sequence analysis and experiments, the L452R kit should have no cross reactivity with other variants, including original Wuhan strain, alpha, beta, gamma, and Omicron BA.1 and BA.2.

CONTENTS

A mix of primers/probe targeting the RNA region coding for S protein (L452R) in SARS-CoV-2 Delta variant genome is provided in a tube as a 20X concentrated working solution. The fluorophore of the probe is HEX™ (Hexachloro-fluorescein, a trademark of Life Technologies, Inc) and the quencher is BHQ-1™ (Black Hole Quencher, a trademark of Biosearch Technologies, Inc.). Users are responsible to provide positive control for the RT-PCR reaction. DNAS can provide an alternative kit that has RPP30 control or that detects L452R in a different fluorescence channel – please inquire with DNAS for more information.

KIT HANDLING AND CONTAMINATION

The DNA Software Assay L452R-Delta 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)
TaqPath™ mastermix (4X)	5
L452R-Delta primers/probe mix (20X)	1
Positive control 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 @ 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.

Delta (HEX™)	Positive Control	Recommended Interpretation
–	–	The PCR reaction failed. Please repeat the experiment
–	+	The sample doesn't contain SARS-CoV-2 Delta RNA.
+	–	The sample contains SARS-CoV-2 Delta RNA. The sample may not contain the positive control RNA
+	+	The sample contains SARS-CoV-2 Delta RNA, and the positive control RNA.

PRE-VALIDATION

Experiments were performed in triplicate using the experimental procedure given above. Limit of detection (LOD) was estimated by performing serial dilution experiments in triplicate (**Figure 1**). For dilution series only one SARS CoV-2 Delta variant template RNA was added. The results show a limit of detection (LOD) < 200 copies/reaction.

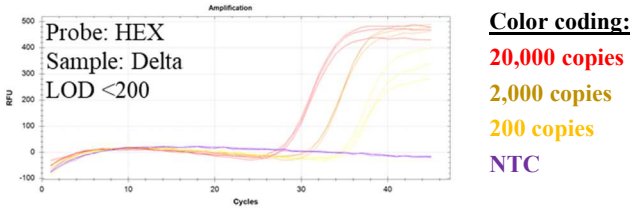

Figure 1: Serial dilution experiments show LOD < 200 molecules for SARS-CoV-2 Delta variant.

Specificity of the kit to SARS-CoV-2 Delta variant was tested previously using QuantiTASE PLUS One-Step RT-qPCR Kit (SKU-KIT-200), Empirical™, with different samples added to each reaction. No positive control was employed since pure standard RNAs were tested in this case. 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 wild type (Twist® Standard RNA #6)

The results of these experiments are shown in **Figure 2** below:

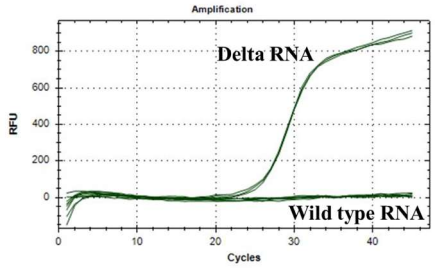

Figure 2: Validation experiments with single targets. Positive signal is only observed with Delta variant RNA that contains the L452R mutation. Baseline background is shown with SARS-CoV-2 wild type RNA.

Conclusion: The data in **Figure 2** indicate that the L452R-Delta assay specifically detects Delta variant but does not detect the wild type. Based on sequence analysis, it can be inferred that the L452R-Delta assay will also not detect earlier VOCs such as Wuhan, alpha, beta, gamma, or new omicron variants BA.1 and BA.2.

CONTACT US

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

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