COVID-19 Mutation RT-qPCR Detection Kit (Alpha, Delta, Kappa)

30 SEP 2021

Catalog Number	Size
QP019M-0050	50 Reactions

Storage Conditions

Stable for up to 1 year at -20°C

Description

Since the onset of the COVID-19 pandemic in early 2020, new genetic variants of concern have been identified and reported in different geographical regions. Genetic changes in the SARS-CoV-2 virus, as universally understood, are significantly associated with its mutability during viral replication and rendering the altered SARS-CoV-2 genetic variants with a competitive advantage. The conferred advantages include but are not limited to conformational changes in the virus spike protein allowing evasion of immune responses and enhanced transmissibility due to stronger binding to target viral ACE2 receptors, for example, on the surface of respiratory tract cells, allowing enhanced entry into the target cell. As a result, the Alpha, Delta, and Kappa variants have spread rapidly across the globe creating concern as to their transmissibility and impact on current vaccines, severity of hospitalization, and degree of mortality.

For advancing the knowledge and competency in assessing the impact and dynamics of the SARS-CoV-2 pandemic, we are committed to providing the research communities across the globe with the highest possible quality, Research-Use-Only molecular assay kit designed for the *in vitro* qualitative detection and differentiation of the mutations in N501Y (Alpha Type) and L452R (Delta/Kappa Type) present in the nasal and nasopharyngeal swab specimens from patients confirmed with known SARS-CoV-2 infection.

Kit Content(s)

2X RT-qPCR MasterMix	500 µl
2X RT-qPCR Mutation MaterMix	500 µl
RScript Enzyme Mix	20 µl
Positive Control	50 µl
Negative Extraction Control	1.0 ml
Nuclease-Free Water	1.0 ml

Required materials but not provided

- A compatible real-time PCR instrument
- Vortex or equivalent
- Microcentrifuge
- Plates and seals for your instruments

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Instrument Compatibility

Thermo QuantStudio™ 5, ABI: 7500 Fast Series; Bio-Rad: CFX96; Roche: LightCycler Series; Agilent: Mx3005p, Qiagen: RotorGene 3000

Reaction Setup

1. Prepare the RT-qPCR Reaction: Thaw and assemble the following components in a 0.2 ml PCR tube on ice just prior to use: 2X RT-qPCR MasterMix/2X RT-qPCR Mutation MaterMix and RScript Enzyme Mix. Caution: Do not add more than one RNA sample into a single qPCR tube. Mix gently. If necessary, centrifuge briefly.

Component	Patient Sample	Patient Sample for Mutation Test	No Template Control (NTC)
RNA Sample	5 µl	5 µl	0 µl
2X RT-qPCR MasterMix	10 µl	0 µl	10 µl
2X RT-qPCR Mutation MasterMix	0 µl	10 µl	0 µl
RScript Enzyme Mix	0.2 µl	0.2 µl	0.2 µl
Nuclease – Free H_2O	4.8 µl	4.8 µl	9.8 µl
Total Volume	20 µl		

* RNA sample including Patient Sample, Patient Sample for Mutation Test, Positive Control and Negative Extraction Control.

* Negative Extraction Control should be extracted by nucleic acid isolation kit before RT-qPCR.

2. Use the Nuclease-free H_2O for the Negative Control while using Positive Control for the Positive Control setup. Cap tubes and place in the thermal cycler.
3. Process in the thermal cycler for 45 cycles as follows:

Steps	Temperature	Time	Cycle(s)
cDNA Synthesis	42°C	15 minutes	1
Pre-Denaturation	95°C	5 minutes	1
Denaturation	95°C	5 seconds	45
Annealing	60°C	30 seconds	
Cooling	40°C	2 minutes	1
Melting curve	40-95°C	0.15°C/sec	1
Cooling	40°C	2 minutes	1

Note: Optimal conditions for amplification will vary depending on the thermal cycler used. It may be necessary to optimize the system for individual primers, template, and thermal cycler.

4. Detection: As two channels (FAM and HEX) are used in this one tube qPCR assay, we recommend to perform the channel calibration as requested by its manufacturer. Please refer to the instrument's user manual to perform this calibration. Choose the FAM and HEX channels for each sample to be tested with the LifeDireX COVID-19 Mutation RT-qPCR Detection Kit (Alpha, Delta, Kappa). Select "None" for ROX passive reference on any qPCR machine requiring ROX as the reference dye.

Expected Performance of Controls and Interpretation of Results

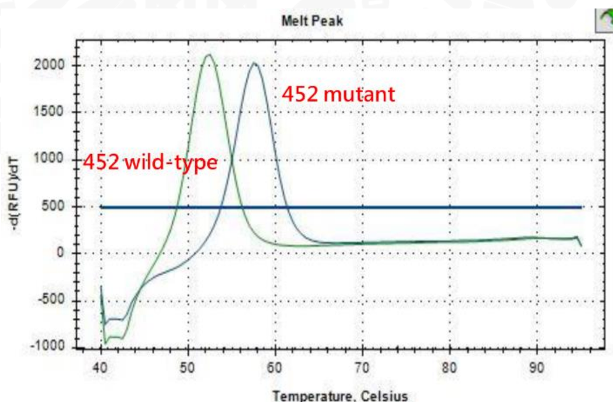
Type	Used to Monitor	Expected Results and Ct Values	
		N (FAM)	RP (HEX)
Positive Control	Flawed assay setup and reagent failure, including degraded primer and probe	Positive Ct ≦ 40.0	Negative ND
No Template Control ("NTC")	Cross-contamination	Negative ND	Negative ND
Negative Extraction Control	Assay or extraction reagent contamination	Negative ND	Positive Ct ≦ 40.0
SARS-CoV-2 Positive	-	Positive Ct ≦ 40.0	+/- Ct ≦ 40.0/ ND
SARS-CoV-2 Negative	-	Negative ND	Positive Ct ≦ 40.0
SARS-CoV-2 Invalid Result	-	Negative ND	Negative ND

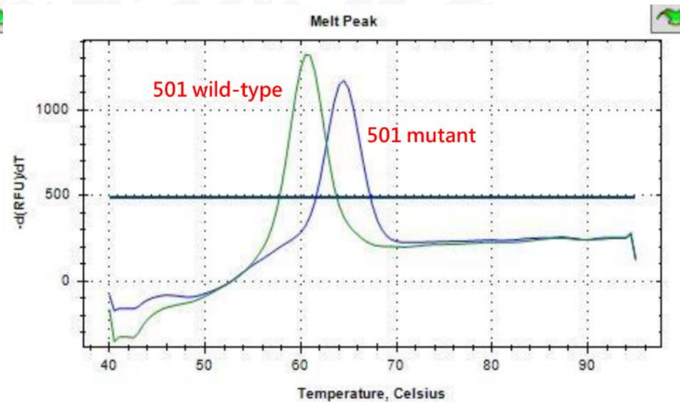
**ND* = Not Detected.

*Results are considered invalid if any control does not perform as specified above.

*Note: The results from this kit should be interpreted in combination with all relevant laboratory findings.

Expected Melting Curve of Mutation Results





L452R wild-type Tm = 52℃ (green line)
L452R mutant Tm = 58℃ (blue line)

N501Y wild-type Tm = 61℃ (green line)
N501Y mutant Tm = 65℃ (blue line)

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Interpretation of Results for SARS-CoV-2 Mutation Test

Mutant	L452R	N501Y
Original	-	-
Alpha	Wild-type	Mutation
Delta	Mutation	Wild-type
Kappa	Mutation	Wild-type

Important notes

1. Shake gently before use to avoid foaming and low-speed centrifugation.
2. Reduce the exposure time.
3. During operation, always wear a lab coat, disposable gloves, and protective equipment.

Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when quantifying nucleic acid targets with the kit.

Trouble	Cause	Solution
Poor Signal or No Signal	Inhibitor Present	1. Perform a dilution series of the PCR template to determine whether the effect of the inhibitory agent can be reduced. 2. Take extra care with the nucleic acid extraction steps to minimize carryover of PCR inhibitors.
	Degraded Template Material	1. Do not store diluted template in water or at low concentrations. 2. Check the integrity of template material by automated or manual gel electrophoresis.
	Inadequate Thermal Cycling Conditions	1. Try using a minimum extension time of 30 sec for genomic DNA and 15 sec for cDNA.
Signal in Negative Control	Contamination of Reaction Components with Target Sequence	1. To minimize the possibility of contamination of PCR components by PCR product or other template, designate a work area exclusively for PCR assay setup. 2. Use a solution of 10% bleach instead of ethanol to prepare the workstation area for PCR assay setup. Ethanol will only induce precipitation of DNA in your work area, while the 10% bleach solution will hydrolyze, as well as dissolve, any residual DNA.

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Poor Reproducibility Across Replicate Samples	Inhibitor Present	1. Perform a dilution series of the PCR template to determine whether the effect of the inhibitory agent can be reduced. 2. Take extra care with the nucleic acid extraction steps to minimize carryover of PCR inhibitors.
	Primer Design	1. Verify primers design at different annealing temperatures.
Low or High Reaction Efficiency	Primer- Dimer	1. Reduce primer concentration. 2. Evaluate primer sequences for complementarity and secondary structure. Redesign primers if necessary. 3. Perform melt-curve analysis to determine if primer- dimers are present.
	Insufficient Optimization	1. Use a thermal gradient to identify the optimal thermal cycling conditions for a specific primer set.

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