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# *PlexPrime*® SARS-CoV-2 L452Q Lambda

Product Code: 7234002
Reactions: 200

**Storage and Stability:**
Reagents are shipped on dry ice or ice packs. All kit components are stable at -25°C to -15°C; refer to expiry on the label. Excessive freeze/thawing is not recommended. Store protected from light at -25°C to -15°C.

**Notes:**
This product is for Research Use Only, not for use in diagnostic procedures.

## RESEARCH USE ONLY

SpeeDx

**Store at -25°C to -15°C**

### Description

The ***Plex****Prime*® SARS-CoV-2 L452Q Lambda assay is an oligo mix designed for single-well RT-qPCR. It targets the RdRp gene and the L452Q mutation in the spike gene of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The reagents are compatible with the following real-time detection systems: Roche LightCycler® 480 Instrument II (LC480 II), the Applied Biosystems® 7500 Fast (7500 Fast), Applied Biosystems® 7500 Fast Dx (7500 Fast Dx), Applied Biosystems® QuantStudio (QuantStudio), and the Bio-Rad CFX96™ IVD (CFX96 IVD) and CFX96 Touch™ (CFX96 Touch) Real-time PCR Detection Systems. It is recommended to be used with the ***Plex****PCR*® Sapphire Core Reagents.

### Components

| Reagents | 200 reactions | Cap colour |
|---|---|---|
| SARS-CoV-2 L452Q Mix, 20x | 2 x 150 µl | Orange |

## Recommended procedures:

### Sample extraction

Samples should be extracted as total nucleic acid (TNA).

### Post extraction setup

1. RT-qPCR Master mix setup 25.0 µl

| Component | Supplied | Volume |
|---|---|---|
| ***Plex*** Mastermix, 2x* | No | 12.5 µl |
| RNase Inhibitor, 50x | No | 0.5 µl |
| RTase, 100x | No | 0.25 µl |
| SARS-CoV-2 L452Q Mix, 20x | Yes | 1.25 µl |
| Nuclease-free water | No | 0.5 µl |
| Total volume (for 1 reaction) | | 15.0 µl |

*Recommended to use ***Plex****PCR*® Sapphire Core Reagents (Cat no 7214002, SpeeDx)

*Recommended to Vortex and centrifuge the components before making up the master mix.*

Add 15.0 µl of the RT-qPCR Master mix to each well.

Add 10.0 µl purified TNA sample to each well.

### Programming and Data Analysis

1. Roche LightCycler® 480 Instrument II (LC480 II)

Refer to LC480 II Instrument Operator's Manual

2. Applied Biosystems® 7500 Fast (7500 Fast), Applied Biosystems® 7500 Fast Dx (7500 Fast Dx)

Refer to Applied Biosystems 7500 FAST/7500 FAST Dx manual

3. Applied Biosystems® QuantStudio (QuantStudio)

Refer to Quantstudio Real-Time PCR Instrument and Flex Real-Time PCR system software manual

4. Bio-Rad CFX96™ IVD (CFX96 IVD) and CFX96 Touch™ (CFX96 Touch)

Refer to CFX96 IVD and CFX96 Touch Real-Time PCR Detection Systems manual

## 2. Instrument Detection Formats

The channels used for LC480 II instrument are shown below.

| Channel | L452Q Mix |
|---|---|
| 533-580 | RdRp |
| 533-610 | L452Q |

The channels used for 7500 Fast and 7500 Fast Dx are shown below.

| Channel | L452Q Mix |
|---|---|
| JOE | RdRp |
| Texas Red | L452Q |

The channels used for QuantStudio are shown below.

| Channel | L452Q Mix |
|---|---|
| VIC/JOE | RdRp |
| ROX/Texas Red | L452Q |

The channels used for CFX96 IVD and CFX Touch are shown below.

| Channel | L452Q Mix |
|---|---|
| HEX | RdRp |
| Texas Red | L452Q |

## 3. Thermocycling Program

Create the following **Cycling program**

- Touch down cycling is for specific amplification of target
- Quantification cycling is for PCR amplification and fluorescence acquisition

| Program Name | Cycles | Target °C | Hold |
|---|---|---|---|
| Reverse Transcriptase | 1 | 48°C | 10 min |
| Polymerase activation | 1 | 95°C | 2 min |
| Touch down cycling: Step down - 0.5°C/Cycle | 10 | 95°C | 5 s |
| | | 61°C – 56.5°C[δ] | 30 s |
| Quantification cycling[+]: Acquisition/Detection | 40 | 95°C | 5 s |
| | | 52°C[+] | 50 s |
| Cooling | 1 | 40°C | 30 s |

[δ] ***Step size:*** *-0.5℃/Cycle,* ***Sec Target:*** *56℃*

[+] ***Analysis mode:*** *Quantification,* ***Acquisition mode:*** *Single*

## 4. Data Analysis

Perform data analysis, as described in the instrument's operator manual, and perform Delta Cq analysis for mutation channels as described below.

***For LC480II SpeeDx Colour Compensation (CC) must be run and applied before analysis.***

*The* ***Plex****PCR® Colour Compensation kit (Cat no 90001, SpeeDx) can be provided upon request, please contact: sales@speedx.com.au*

***Delta Cq method***

*In mutation channels, wild-type sequence may result in non-specific signals, especially when the viral load is high. Differentiation between detection of wild-type or mutant sequence is based on a Delta Cq method where:*

*ΔCq = Mut Cq – RdRp Cq*

*A true mutant signal falls within a specific delta Cq range; these ranges should be established by the user for each mutation by testing titrations of known wild-type and mutant samples. An example of a delta Cq range is provided in the table below.*

| Target | Cq | Result |
|---|---|---|
| RdRp | POS | SARS-CoV-2 detected |
| L452Q | NEG | |
| RdRp | POS | SARS-CoV-2 L452Q mutation detected |
| L452Q | POS (ΔCq<5)* | |
| RdRp | NEG | SARS-CoV-2 not detected |
| L452Q | NEG | |
| RdRp | NEG | Invalid |
| L452Q | POS | |

*An example of a delta Cq cut-off in a specified channel; note that this value isa recommendation only, and a true range should be derived experimentally using known samples.