PlexPrime® SARS-CoV-2 P681R Delta

Product Code: 7154002
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

Store at -25°C to -15°C

Description

The *PlexPrime®* SARS-CoV-2 Delta P681R is an oligo mix designed for single-well RT-qPCR. It targets the RdRp gene and the P681R 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 use with the *PlexPCR®* Sapphire Core Reagents.

Components

Reagents	200 reactions	Cap colour
SARS-CoV-2 P681R Mix, 20x	2 x 150 µl	Blue

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 P681R Mix, 20x	Yes	1.25 µl
Nuclease-free water	No	0.5 µl
Total volume (for 1 reaction)		15.0 µl

*Recommended to use *PlexPCR®* 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)
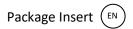
   Refer to Applied Biosystems 7500 FAST/7500 FAST Dx manual

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	P681R Mix
533-580	RdRp
618-660	P681R

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

Channel	P681R Mix
JOE	RdRp
Cy5	P681R

The channels used for QuantStudio are shown below.

Channel	P681R Mix
VIC	RdRp
Cy5	P681R

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

Channel	P681R Mix
HEX	RdRp
Cy5	P681R

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°C/Cycle, **Sec Target:** 56°C*

+ *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 Delta Cq method where:

ΔCq = Mut Cq – RdRp Cq

A true mutant signal falls within a specific delta Cq range; these ranges shall be established by the user for each mutation from testing titrations of known wild-type and mutant samples. Examples of such ranges i.e. Delta Cq cut-offs are provided in the table below.

Target	Cq	Result
RdRp	POS	SARS-CoV-2 detected
P681R	NEG	
RdRp	POS	SARS-CoV-2 P681R mutation detected
P681R	POS (ΔCq<6)*	
RdRp	NEG	SARS-CoV-2 not detected
P681R	NEG	
RdRp	NEG	Invalid
P681R	POS	

*An example of Delta Cq cut-offs in a specified channel; note that these are recommendations only, and a true range shall be derived experimentally using known samples.