Package Insert EN

PlexPrime® SARS-CoV-2 Alpha/Beta/Gamma+

Product Code: 7144002
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 Alpha/Beta/Gamma+ is an oligo mix designed for single-well RT-qPCR. It targets the RdRp gene and 3 mutations in the spike gene, N501Y, S982A and E484K 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™ Dx (CFX96 Dx) and CFX96 Touch™ (CFX96 Touch) Real-time PCR Detection Systems. It is recommended to use with the ***Plex****PCR*® Sapphire Core Reagents.

Components

Reagents	200 reactions	Cap colour
SARS-CoV-2 var(3) Mix, 20x	2 x 150 µl	Purple

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 var(3) 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™ Dx (CFX96 Dx) and CFX96 Touch™ (CFX96 Touch)

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

2. Instrument Detection Formats

The channels used for LC480 II instrument are shown below.

Channel	CoV-2 Mix
465-510	E484K
533-580	RdRp
533-610	S982A
618-660	N501Y

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

Channel	CoV-2 Mix
FAM	E484K
JOE	RdRp
Texas Red	S982A
Cy5	N501Y

The channels used for QuantStudio are shown below.

Channel	CoV-2 Mix
FAM	E484K
VIC	RdRp
ROX	S982A
Cy5	N501Y

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

Channel	CoV-2 Mix
FAM	E484K
HEX	RdRp
Texas Red	S982A
Cy5	N501Y

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.

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*

Target	Cq	Result
RdRp	POS	SARS-CoV-2 detected
E484K	NEG	
S982A	NEG	
N501Y	NEG	
RdRp	POS	SARS-CoV-2 Variant(s) detected (if one or more mutations are positive)
E484K	POS (ΔCq -2 to 5)*	
S982A	POS (ΔCq -5 to 5)*	
N501Y	POS (ΔCq -2 to 5)*	
RdRp	NEG	SARS-CoV-2 not detected
E484K	NEG	
S982A	NEG	
N501Y	NEG	
RdRp	NEG	Invalid (where one or more mutations are positive)
E484K	POS	
S982A	POS	
N501Y	POS	

*Delta Cq cut-off (Variant Cq – RdRp Cq)