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⦿ Aegea Biotechnologies rapid PCR SARS-CoV-2 test (high sensitivity & specificity; able to detect different strain types)

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ABSTRACT

This protocol is for the Aegea Biotechnologies rapid PCR-based SARS-CoV-2 test. This assay uses patented "Switch-Blocker" technology as well as taqman probes to test for presence of SARS-CoV-2 and simultaneously orthogonally validate. A single amplification reaction is performed, and "Switch Blocker" is used on the forward strand and taqman is used on the reverse strand. The assay design has high sensitivity & specificity--single nucleotide level. Moreover, it is able to detect the SARS-CoV-2 L strain vs the SARS-CoV-2 S strain. The test can be adapted to point of care (Roche LIAT) as well as for different SARS-CoV-2 strains as the virus mutates. A next generation version of the assay could identify the presence of the SARS-CoV-2 L/S strains vs. influenza A/B. Finally, because of the sensitivity and specificity, the Aegea PCR-based SARS-CoV-2 test should be able to use saliva samples, and it is suitable for pooled testing. This protocol is designed for high throughput PCR (96 or 384 well plate formats).

Keywords: PCR, COVID-19, coronavirus, SARS-CoV-2, high throughput, multiplex, Switch-Blocker, taqman, high sensitivity, high specificity, accurate, pooling, saliva, strain types, L-strain, S-strain, combination SARS-CoV-2 and influenza

EXTERNAL LINK

<http://www.aegeabiotech.com>

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STEPS MATERIALS

NAME	CATALOG #	VENDOR
RNaseP_FP1	RNaseP_FP1	
AEGEA 1001B_Taq	AEGEA 1001B_Taq	
AEGEA 1001RP	AEGEA 1001RP	
AEGEA_1003AF_Taq	AEGEA_1003AF_Taq	
RNaseP_RP1	RNaseP_RP1	
TaqPath™ 1-Step RT-qPCR Master Mix	A15300	
AEGEA 1001B_Switch-Blocker	AEGEA 1001B	
AEGEA 1002B_Taq	AEGEA 1002B_Taq	
AEGEA 1001cFP	AEGEA 1001cFP	
Nuclease-free Water	AM9937	
gblock L-type	gblock L-type	
gblock S-type	gblock S-type	

EQUIPMENT

NAME	CATALOG #	VENDOR
QuantStudio 5	A34322	

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1 Prepare Reaction Mix

10ul of the isolated total RNA will be used in a 20ul reaction with TaqPath™ 1-Step RT-qPCR Master Mix (5ul TaqPath Mastermix and 5ul of an oligo mix containing

- 0.5uM AEGEASwitch-Blocker (FAM),
- 0.5uM AEGEA COVID Forward Primer C,
- 2uM AEGEA COVID Reverse Primer,
- 0.8uM AEGEA L-Strain Probe (VIC),
- 0.8uM AEGEA S-Strain Probe (NED),
- 0.5uM AEGEARNaseP Forward Primer
- 0.5 uM AEGEA RNaseP Reverse Primer
- 0.8uM AEGEA RNaseP Probe Alexa 647




TaqPath™ 1-Step RT-qPCR Master Mix
Catalog #: A15300

 AEGEA 1001B_Switch-Blocker
Catalog #: AEGEA 1001B


 0.5 µl

 AEGEA 1001cFP
Catalog #: AEGEA 1001cFP


 0.5 µl

 AEGEA 1001RP
Catalog #: AEGEA 1001RP

 2 µl

 RNaseP_FP1
Catalog #: RNaseP_FP1


 0.5 µl

 RNaseP_RP1
Catalog #: RNaseP_RP1


 0.5 µl

 AEGEA_1003AF_Taq
Catalog #: AEGEA_1003AF_Taq

 0.8 µl

 AEGEA 1001B_Taq
Catalog #: AEGEA 1001B_Taq

 0.8 µl


 AEGEA 1002B_Taq
Catalog #: AEGEA 1002B_Taq

0.8 µl


Table: Reaction components and final concentrations

Component	Final Conc.
Nuclease	
Free H ₂ O	
TaqPath™	
1-Step RT-qPCR Master Mix (4x)	
AEGEA Switch-Blocker(FAM)	0.5 µM
AEGEA COVID	0.5 µM
Forward Primer	
AEGEA COVID	2 µM
Reverse Primer	
AEGEA	0.5 µM
RNaseP Forward Primer	
AEGEA	0.5 µM
RNaseP Reverse Primer	
AEGEA	0.8 µM
RNaseP Probe Alexa 647)	
AEGEA COVID	0.8 µM
L-Strain Probe (VIC)	
AEGEA COVID	0.8 µM
S-Strain Probe (NED)	
RNaseP	Variable
Template (RNA-L/RNA-S)	Variable

- 2 10ul nuclease free water (NTC) or 10ul (combined) gBlock control L/S (5 uL gBlock control L; 5uL gBlock control S) will be run in parallel as negative and positive controls respectively.

**Nuclease-free Water**
Catalog #: AM9937

10 µl

**gblock L-type**
Catalog #: gblock L-type

5 µl



gblock S-type

Catalog #: gblock S-type

5 µl

- 3 The oligo mix also contains reagents for an internal adequacy control which targets the human RNaseP RNA transcript.



QuantStudio 5

qPCR

ThermoFisher

A34322



Table:qPCR Cycling Condition

Cycle Step	Temperature 0C	Time	Cycles	
Step 1	25°C	2 min	1x	
RT Reaction	50°C	15 min	1x	
Initial Polymerase Activation and DNA Denaturation	95°C	2 min	1x	
Denaturation	95°C	3 sec	45 X	
Annealing	65°C	10 sec		
Annealing	52 0C	10 sec	Detection	
Extension	58 0C	1 min		
Melt Curve	95°C	15 sec	1x	
	40°C	15 sec	1x	Detection During Ramp From 40 0C to 95 0C
	95°C	1 sec	1x	