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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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Protocol status: In development
We are still developing and optimizing this protocol

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Keywords: rapid pcr sar, aegea biotechnologies rapid pcr, different sar, presence of sar, aegea biotechnology, presence of the sar, based sar, aegea pcr, coronavirus, combination sar, sar, next generation version of the assay, virus mutate, rapid pcr, assay design

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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

Materials

STEP MATERIALS

- RNAseP_FP1 **Catalog #**RNAseP_FP1
- AEGEA 1001B_Taq **Catalog #**AEGEA 1001B_Taq
- AEGEA 1001RP **Catalog #**AEGEA 1001RP
- AEGEA_1003AF_Taq **Catalog #**AEGEA_1003AF_Taq
- RNAseP_RP1 **Catalog #**RNAseP_RP1
- TaqPath™ 1-Step RT-qPCR Master Mix **Catalog #**A15300
- AEGEA 1001B_Switch-Blocker **Catalog #**AEGEA 1001B
- AEGEA 1002B_Taq **Catalog #**AEGEA 1002B_Taq
- AEGEA 1001cFP **Catalog #**AEGEA 1001cFP
- Nuclease-free Water **Catalog #**AM9937
- gblock L-type **Catalog #**gblock L-type
- gblock S-type **Catalog #**gblock S-type

Protocol materials

- gblock S-type **Catalog** #gblock S-type
- RNAseP_RP1 **Catalog** #RNAseP_RP1
- TaqPath™ 1-Step RT-qPCR Master Mix **Catalog** #A15300
- AEGEA 1001cFP **Catalog** #AEGEA 1001cFP
- Nuclease-free Water **Catalog** #AM9937
- AEGEA 1001B_Switch-Blocker **Catalog** #AEGEA 1001B
- AEGEA 1002B_Taq **Catalog** #AEGEA 1002B_Taq
- RNAseP_FP1 **Catalog** #RNAseP_FP1
- AEGEA 1001B_Taq **Catalog** #AEGEA 1001B_Taq
- gblock L-type **Catalog** #gblock L-type
- AEGEA 1001RP **Catalog** #AEGEA 1001RP
- AEGEA_1003AF_Taq **Catalog** #AEGEA_1003AF_Taq

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
a. 0.5uM AEGEASwitch-Blocker (FAM),
b. 0.5uM AEGEA COVID Forward Primer C,
c. 2uM AEGEA COVID Reverse Primer,
d. 0.8uM AEGEA L-Strain Probe (VIC),
e. 0.8uM AEGEA S-Strain Probe (NED),
f. 0.5uM AEGEARNaseP Forward Primer
g. 0.5 uM AEGEA RNaseP Reverse Primer
h .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 H2O	
	TaqPath™ 1-Step RT-qPCR Master Mix (4x)	
	AEGEA Switch-Blocker(FAM)	0.5 uM
	AEGEA COVID Forward Primer	0.5 uM
	AEGEA COVID Reverse Primer	2 uM
	AEGEA RNaseP Forward Primer	0.5 uM
	AEGEA RNaseP Reverse Primer	0.5 uM
	AEGEA RNaseP Probe Alexa 647)	0.8 uM
	AEGEA COVID L-Strain Probe (VIC)	0.8 uM
	AEGEA COVID S-Strain Probe (NED)	0.8 uM
	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.

Equipment

QuantStudio 5	NAME
qPCR	TYPE
ThermoFisher	BRAND
A34322	SKU
https://www.thermofisher.com/us/en/home/life-science/pcr/real-time-pcr/real-time-pcr-instruments/quantstudio-3-5-real-time-pcr-system.html	LINK

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	Detectio n	
	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	