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

Lyle J. Arnold Ph.D., Stella M. Sung Ph.D.¹

¹Aegea Biotechnologies, Inc.

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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

DOI

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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

- 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





Table:Reaction components and final concentrations

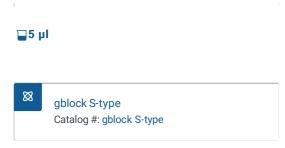
Component	Final Conc.
Nuclease	
Free H20	
TaqPath™	
1-Step RT-qPCR Master Mix (4x)	
AEGEA Switch-Blocker(FAM)	0.5 uM
AEGEA COVID	0.5 uM
Forward Primer	
AEGEA COVID	2 uM
Reverse Primer	
AEGEA	0.5 uM
RNaseP Forward Primer	
AEGEA	0.5 uM
RNaseP Reverse Primer	
AEGEA	0.8 uM
RNaseP Probe Alexa 647)	
AEGEA COVID	0.8 uM
L-Strain Probe (VIC)	
AEGEA COVID	0.8 uM
S-Strain Probe (NED)	
RNAseP	Variable
Template	Variable
(RNA-L/RNA-S)	

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.



□10 μl





⊒5 μl

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

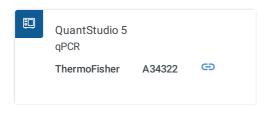


Table:qPCR Cycling Condition

Cycle Step	Temperature 0C	Time	Cycles	
Step 1	25°C	2 min	1x	
RT Reaction	50°C	15 min	1x	
Initial Polymerase	95°C	2 min	1x	
Activation and DNA				
Denaturation				
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 40°C 95°C	95°C	15 sec	1x	
	15 sec	1x	Detection	
			During Ramp From 40	
				0C to 95 0C
	95°C	1 sec	1x	