Mikro SARS-CoV-2 Multiplex Protocol V.1

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[1]Mikro

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MATERIALS

NAME	CATALOG #	VENDOR
Ethanol, Absolute, Molecular Biology Grade	BP2818500	Thermo Fisher Scientific
Buffer AVL	19073	Qiagen
Buffer AVE	1020953	Qiagen
UltraPlex 1-Step ToughMix (4X)	95166-01K	Quantabio
Nuclease-Free Water	129117	Qiagen

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Ethanol, Absolute, Molecular Biology Grade	BP2818500	Thermo Fisher Scientific
Buffer AVE	1020953	Qiagen
Buffer AVL	19073	Qiagen
UltraPlex 1-Step ToughMix (4X)	95166-01K	Quantabio

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BEFORE STARTING

The protocol is designed for a single sample, utilizing a single well.
It is designed for a custom RT-qPCR device, but may be adapted for other equipment.

Sample Collection

1 Remove the lid of sample collector. Direct the sample provider to expel saliva into the collector until 0.5 mL has been collected. Replace lid.

Teeth brushing, mouthwash, drinks, food, and nasal sprays should be avoided for half an hour before sample collection.

Heat Pre-Treatment

2 Load sample for RNA extraction.

2.1 Apply heat-treatment to sample

Step	Action	Specification	Time
1	Heat	95°C	2 min
2	Cool	Room temperature	2 min
3	Centrifuge	500 x g	2 min

2.2 Collect cellular debris.
Add **100 µl** of

Ethanol, Absolute, Molecular Biology Grade
by Thermo Fisher Scientific
Catalog #: BP2818500

Mix for **00:00:30**

RNA Extraction

3 Immediately proceed with RNA extraction. Do not store sample.

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3.1 Prepare **500 µl** of

Buffer AVL
by Qiagen
Catalog #: 19073

3.2 Add **100 µl** of heat-treated sample to buffer.
Mix for **00:00:15**

3.3 Incubate at room temperature for **00:07:00**

3.4 Centrifuge at 500 x g for **00:02:00**
Then remove buffer.

4 Proceed with RNA elution.

4.1 Add **60 µl** of

Buffer AVE
by Qiagen
Catalog #: 1020953

4.2 Centrifuge at 1000 x g for **00:02:00**

RT-qPCR

5 Prepare **8 µl** multiplex primer-probe-water mix in advance, containing the following:

Component	Spec	Concentration	Volume (1 reaction)
2019-nCoV_N1-F	US CDC	200nM	0.6 µL
2019-nCoV_N1-R	US CDC	400nM	1.2 µL
2019-nCoV_N1-P	US CDC	200nM	0.6 µL
2019-nCoV_N2-F	US CDC	200nM	0.6 µL
2019-nCoV_N2-R	US CDC	400nM	1.2 µL
2019-nCoV_N2-P*	Custom probe (JUN-QSY)	200nM	0.6 µL

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RP-F	US CDC	200nM	0.6 µL
RP-R	US CDC	400nM	1.2 µL
RP-P*	Custom probe (VIC-MGB)	200nM	0.6 µL
Nuclease-free water			0.8 µL

6 Prepare **140 µl** of master mix in advance, using

UltraPlex 1-Step ToughMix (4X)
by Quantabio
Catalog #: 95166-01K

with the following quantities:

Component	Volume (1 reaction)
UltraPlex 1-Step ToughMix	15 µL
Primer-probe-water mix	8 µL
Nuclease-free water	117 µL

7 Add **60 µl** of previously purified RNA sample to **140 µl** of master mix.
Load onto qPCR device.

8 Proceed with thermocycling, under the following conditions:

Step	Temperature	Time
1	50°C	10 min
2	95°C	1 min
3	95°C	3 sec
4	60°C	30 sec
Repeat 40 Cycles for Step 3-4		

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