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Mikro SARS-CoV-2 Multiplex Protocol V.1

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

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Disclaimer

Materials

MATERIALS

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

STEP MATERIALS

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

Protocol materials

- UltraPlex 1-Step ToughMix (4X) **Quantabio Catalog #**95166-01K
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Before start

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.

> Note
>
> 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 **Thermo Fisher Scientific Catalog #**BP2818500

Mix for 00:00:30

RNA Extraction

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

3.1 Prepare 500 µL of

Buffer AVL **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 **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
	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) **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		