

Sep 10, 2020

Version 1

Mikro SARS-CoV-2 Multiplex Protocol V.1

DOI

<https://dx.doi.org/10.17504/protocols.io.bk8dkzs6>

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Protocol Citation: Toby Overmaat 2020. Mikro SARS-CoV-2 Multiplex Protocol. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bk8dkzs6>

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Protocol status: In development

We are still developing and optimizing this protocol

Created: September 10, 2020

Last Modified: September 10, 2020

Protocol Integer ID: 41957

Keywords: sar, protocol

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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**

⊗ Ethanol, Absolute, Molecular Biology Grade **Thermo Fisher Scientific Catalog #BP2818500**

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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		