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Isolation of SARS-Cov2 RNA from Humans Without High Demand Reagents

In 1 collection

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1 Works for me dx.doi.org/10.17504/protocols.io.be8ujhww

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ABSTRACT

Viral RNA isolation kits used in PCR based tests for the novel coronavirus (SARS-Cov2) are in short supply. Our group sought to identify a method to isolate viral RNA without the use of a kit or other supplies in high demand by clinical labs. Using a TRIzol based RNA extraction, followed by a glycogen precipitation, we are able to isolate a sufficient quality and quantity of RNA for quantitative PCR, and/or droplet digital PCR. This method has been confirmed to work as well as a viral RNA isolation kit, shown to work with SARS-Cov2 RNA-spiked into human samples, and in detecting SARS-Cov2 in known positive cases of COVID-19.

GUIDELINES

Samples should be processed for RNA extraction (at least up until they can be frozen at -80 ºC) within 48 hours of collection.

MATERIALS TEXT

- TRIzol (Ambion 15596026)
- GlycoBlue (Thermo Fisher #AM9515)
- 100% Isopropanol
- 100% Chloroform
- 80% ethanol
- Nuclease Free Water
- DNase/RNase free 1.5 mL microcentrifuge tubes
- Phasemaker tubes (Invitrogen A33248)

SAFETY WARNINGS

Human samples should be handled with care, and sample prepartion performed in at least a BSL-2 lab.

1 Transfer the swab into a DNase/RNase free 1.5 mL microcentrifuge tube.
•NOTE: The swabs we use can be pulled off their stick for easier processing.

2 Add 500 µL of TRIzol to the microcentrifuge tube.

3 Close tube and shake for 2 min. by hand.

4 Remove the swab and briefly centrifuge.
•NOTE: The swab should be removed from the TRIzol, and squeezed against the side of the tube to retain as much sample as possible.

4.1 Samples can be frozen at -80 ºC at this point if needed, then thawed for use when ready.

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5 Centrifuge Phasemaker tubes (Invitrogen A33248) for 30 s at 16,000xg

5.1 If Phasemaker tubes are not available, substitute the steps below for steps 6-11:
1)Add 100 uL of 100% chloroform.
2)Vortex for 30 s.
3)Incubate for 3 min. at room temperature.
4)Centrifuge at 12,000 x g for 10 min. at 4 ºC.
5)Transfer supernatant to a new DNase/RNase free 1.5 mL microcentrifuge tube.
6) Continue with protocol at step 7, adding 250 µL of 100% isopropanol to each tube.

6 Transfer the entire sample to a Phasemaker tube and incubate at RT for 5 min

7 Add 100 uL of 100% chloroform to the phasemaker tube.

8 Shake the tube for 15 seconds by hand (DO NOT VORTEX).

9 Incubate for 10 min. at room temperature.

10 Centrifuge for 5 min. at 16,000xg at 4°C.

11 Transfer the aqueous phase (clear) to a new DNase/RNase free 1.5 mL microcentrifuge tube, and dispose of the Phasemaker tube.

12 Add 250 µL of 100% isopropanol

13 Add 2 µL of GlycoBlue coprecipitant

14 Vortex for 30 s.

15 Centrifuge at 12,000 x g for 20 min. at 4 ºC.

16 Discard the supernatant, keep the pellet.

17 Add 80% ethanol to wash the pellet.

18 Immediately remove all of the ethanol.
•NOTE: Once most of the ethanol is removed, the tubes can be briefly centrifuged and a pipet used to remove as much of the remaining ethanol as possible.

19 Allow pellet to dry for 3 min. at room temperature.

20 Resuspend pellet in nuclease free water.

20.1 For both droplet digital PCR and qPCR protocols, 25µL nuclease free water is an appropriate starting volume, but can be adjusted if needed.

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