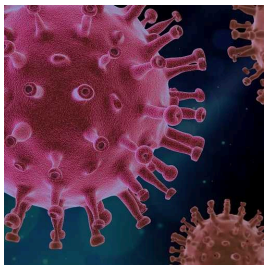
Apr 21, 2020

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



In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.be8ujhww

Joseph Patterson[1], Allyson Cole-Strauss[1], John Beck[1], Caryl Sortwell[1], Jack Lipton[1]

[1]Michigan State University

Coronavirus Method De...

Joseph Patterson
Michigan State University

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Protocol Citation: Joseph Patterson, Allyson Cole-Strauss, John Beck, Caryl Sortwell, Jack Lipton 2020. Isolation of SARS-Cov2 RNA from Humans Without High Demand Reagents. **protocols.io https://dx.doi.org/10.17504/protocols.io.be8ujhww**

Protocol status: Working
We use this protocol and it's working

Created: April 17, 2020

Last Modified: December 11, 2020

Protocol Integer ID: 35828

Keywords: RNA extraction, COVID-19, SARS-Cov2, Limited supplies, Alternative reagents, viral rna isolation kit, cov2 rna from human, tests for the novel coronavirus, high demand reagents viral rna isolation kit, cov2 rna, novel coronavirus, viral rna without the use, based rna extraction, rna extraction, viral rna, cov2, isolation of sar, detecting sar, quantity of rna, rna, quantitative pcr, digital pcr, pcr, isolate, droplet digital pcr,

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

- 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)

Troubleshooting

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.

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.