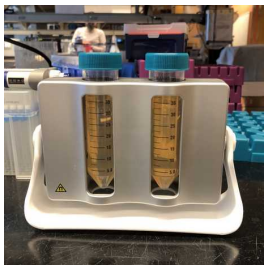
Jan 21, 2022

# Manual Nanotrap Concentration and RNA Extraction for SARS-CoV-2 Viral Capture



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**We use this protocol and it's working**

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## Abstract

This protocol details a method for SARS-CoV-2 capture and concentration through the use of Nanotrap® Magnetic Virus Particles from 40mL of wastewater sample.

## Image Attribution

Fisher Scientific

# Materials

### Equipment

- pH probe
- Magnetic rack (Dynamag)
- Centrifuge
- QIAamp Viral RNA Mini Kit (Cat. No. 52904 or 52906)
- Conical tube (50mL)
- Eppendorf Research Plus Single Channel Pipette
- LabGard Biological Safety Cabinet - Class 2 A2 Biosafety Cabinet
- Autoclave - Amsco Lab 240 Steam Sterilizer

### Materials (per sample)

- 10 µL of BRSV
- 400 µL of Nanotrap Magnetic Virus Particles (10) **Ceres Nano Catalog #**44202
- 1mL of Molecular Grade Water **ATCC Catalog #**60-2450
- Three 1.7mL tubes
- 40 µL of 1X PBS (Phosphate-buffered saline )
- 560 µL of Buffer AVL **Qiagen Catalog #**19073
- 5.6 µL of Carrier RNA **Thermo Fisher Catalog #**4382878
- 560 µL of 96% ethanol
- 500 µL of Buffer AW1 **Qiagen Catalog #**19081
- 500 µL of Buffer AW2 **Qiagen Catalog #**19072
- 60 µL of Buffer AVE **Qiagen Catalog #**1020953

## Protocol materials

- Carrier RNA **Thermo Fisher Catalog #**4382878
- Buffer AW1 **Qiagen Catalog #**19081
- Buffer AW2 **Qiagen Catalog #**19072
- Buffer AVE **Qiagen Catalog #**1020953
- Nanotrap Magnetic Virus Particles (10) **Ceres Nano Catalog #**44202
- Molecular Grade Water **ATCC Catalog #**60-2450
- 1X PBS (Phosphate-buffered saline )
- Buffer AVL **Qiagen Catalog #**19073
- Ethanol **P212121 Catalog #**BE-BDH1156
- double distilled water (ddH2O)

## Concentration Procedure

1 Place a 50 mL conical tube in a tube rack.

2 Vigorously shake the sample to re-suspend solids. Immediately move on to Step 3.

3 Add 50 mL of wastewater to the conical tube inside the biosafety cabinet.

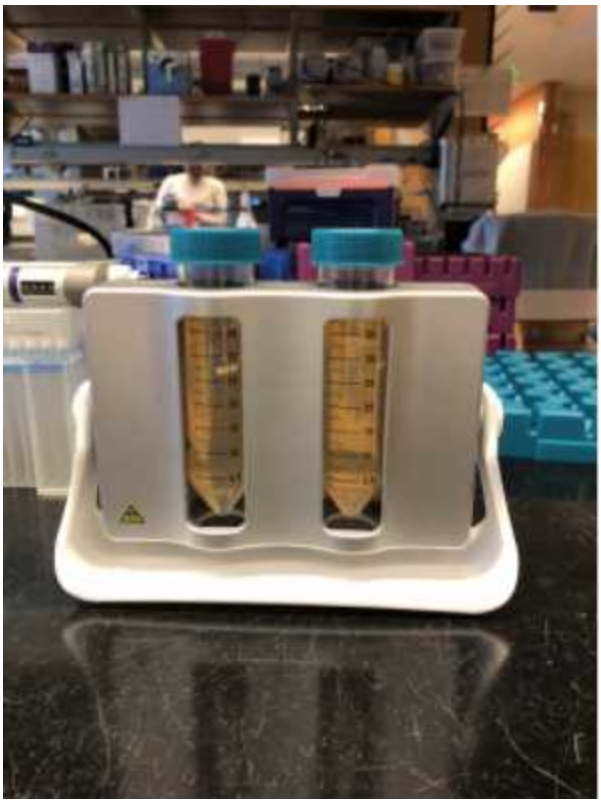
4 Transfer 40 mL of the top supernatant into a new conical tube.

5 Add 10 µL of BRSV to the conical tube.

6 Add 400 µL of Nanotrap Magnetic Virus Particles (10) **Ceres Nano Catalog #**44202 to the sample and invert 2-3 times to mix together and to create a 10:1 sample volume to particle ratio.

7 Invert samples 3-4 times every 5 minutes at Room temperature for 00:20:00 . 20m

8 Use a magnetic rack to separate the magnetic Nanotrap particles from the sample. Allow the sample to sit in the rack for at least 00:10:00 . 10m

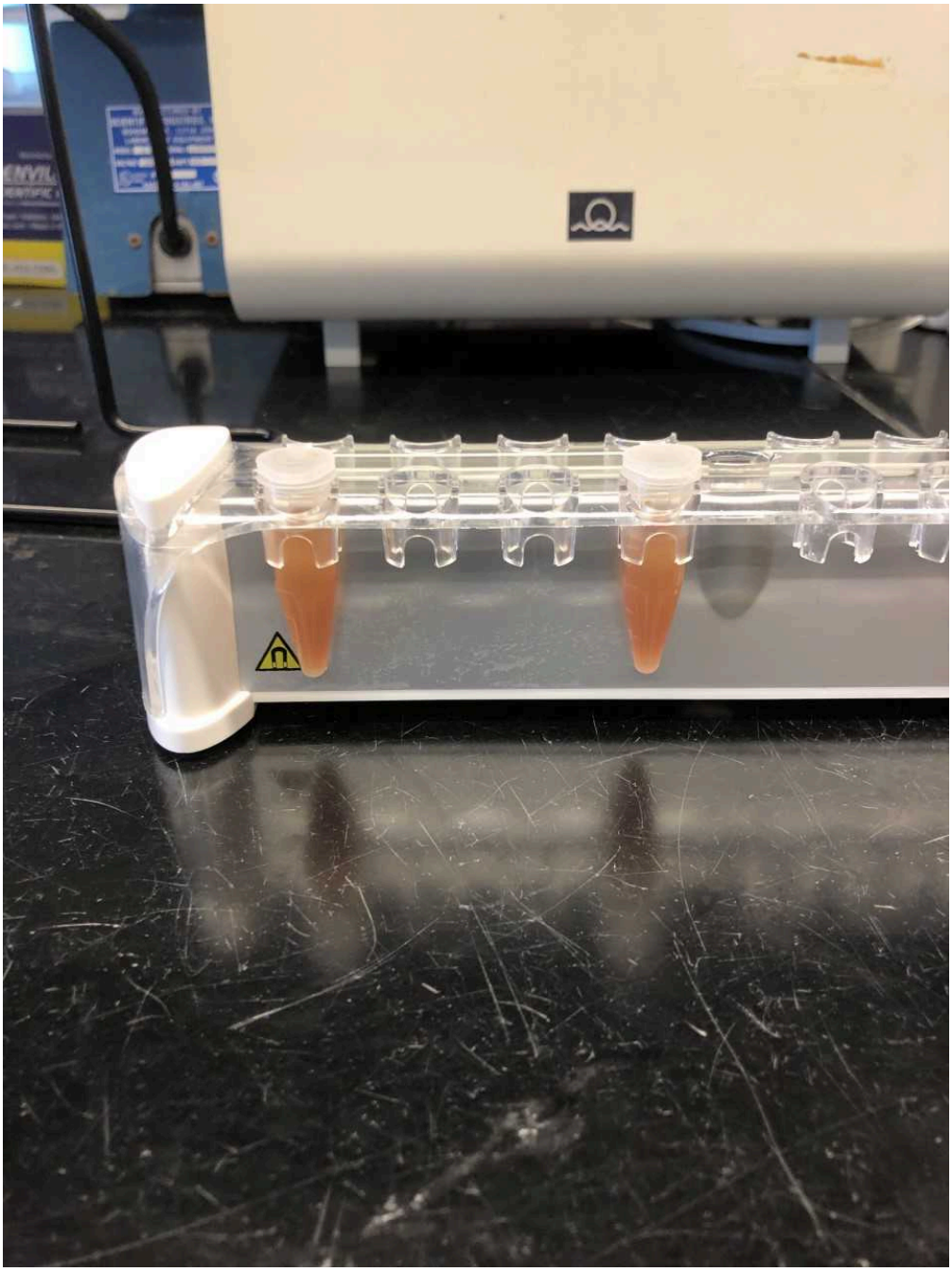


Samples must sit in a magnetic rack for at least 10 minutes to allow for particle separation.

9 Use a pipette to discard the supernatant. Be careful not to disturb the red pellet of Nanotrap particles.

10 Add 1 mL of Molecular Grade Water **ATCC Catalog #**60-2450 to the conical tube. Vortex the tube to re-suspend the pellet.

11 Transfer liquid to a 1.7mL tube. Place tubes on magnetic rack and allow to sit for 00:02:00 . 2m



Sample in 1.7 mL tube sits in a magnetic rack for 2 minutes.

A red pellet of Nanotrap particles congregates to the magnetic side of the 1.7 mL tube.

12 Remove and discard the supernatant. Do not disturb the pellet.

13 Add 140 µL of 1X PBS to the particle pellet.

Note

See "Appendix I: Preparing Buffer Solution (1X PBS)" for instructions on how to prepare 1X PBS.

14 In a separate 1.7 mL tube, add 560 µL of Buffer AVL **Qiagen Catalog #**19073 and 5.6 µL of Carrier RNA **Thermo Fisher Catalog #**4382878. Mix well by pipetting up and down several times.

15 In a regular, non-magnetic rack, add the Buffer AVL and carrier RNA to the particle pellet. Suspend the pellet by using the pipette to wash the sides of the conical tube with the lysis buffer, carrier RNA, and 1X PBS mixture.

16 Incubate the sample at Room temperature for 00:10:00. 10m

17 Use a magnetic rack to separate the magnetic Nanotrap particles from the sample. Allow sample to sit on the rack for 00:02:00. 2m

18 Transfer the supernatant to a 1.7 mL tube and discard the pellet.

## Extraction Step (Qiagen Viral RNA Minikit) 11m

19 Add 560 µL of 96% Ethanol **P212121 Catalog #**BE-BDH1156 to sample. Mix well by pipetting up and down several times.

20 Add 630 µL of the sample mix onto a QIAamp Mini column.

21 Centrifuge sample at full speed for 00:01:00. Once complete, discard the filtrate. 1m

22 Repeat Step 26 until all of the sample has gone through the column.

23 Transfer the QIAamp Mini Column into a new collection tube, and discard the tube containing the filtrate.

24 Open the QIAamp Mini Column and add 500 µL of Buffer AW1 **Qiagen Catalog #**19081. Centrifuge the sample at full speed for 00:01:00. Once complete, discard the filtrate. 1m

25 Open the QIAamp Mini Column and add 500 µL of Buffer AW2 **Qiagen Catalog #**19072. Centrifuge the sample at full speed for 00:03:00. Once complete, discard the filtrate. 3m

26 Place the QIAamp Mini Column into a new collection tube and discard the old one containing filtrate. Centrifuge the sample at full speed for 00:01:00 to remove any buffer AW2 carryover. 1m

27 Place the QIAamp Mini Column into a final, labeled tube and discard the old one containing the filtrate

28 Open the QIAamp Mini Column and add 60 µL of Buffer AVE **Qiagen Catalog #**1020953 and incubate at Room temperature for 00:03:00. 3m

29 Centrifuge the sample at 16000 rpm for 00:02:00. 2m

30 Separate sample into at least two aliquots. Store in -80 °C freezer until it is ready for PCR.

## Appendix I: Preparing Buffer Solution (1X PBS)

31 Begin making the 10X PBS Solution by mixing the following:

- 80 g NaCl
- 2 g KCl
- 14.4 g $Na_2HPO_4$

- 2.4 g $KH_2PO_4$
- 800 mL Ddwater

32 Adjust the solution to pH 7.4 by adding 5% NaOH and/or 5% HCl.

Note

- Swirl the mixture after adding either NaOH or HCl.
- Use a pH probe in between adding NaOH or HCl to see if the pH has reached 7.4 yet.
- Wash off tip of probe with DI water in between uses.

33 Add more double distilled water (ddH2O) until the volume is 1000 mL.

34 Dilute the PBS solution by adding 100 mL of 10X PBS to 900 mL distilled water to create 1X PBS.

35 Dispense mixture into aliquots and autoclave for 00:20:00. Store at room temperature.