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We are still developing and optimizing this protocol

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Protocol for Isolation of total RNA from Wastewater

Forked from Protocol for Safe, Affordable, and Reproducible Isolation of SARS-CoV-2 RNA from Wastewater

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Coronavirus Method Development Community | Davida's workspace | 4 more workspaces

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ABSTRACT

This protocol describe the workflow for fractioning wastewater and performing RNA isolation from each fraction.

GUIDELINES

1. Use good lab practices at all times.
2. Prior to performing any procedure, ensure that proper PPE is donned.
3. Clean all work surfaces and instruments (i.e., pipettes, vortexes, centrifuges) with 10% bleach followed by 70% ethanol.
4. Work with wastewater in a biosafety cabinet inside a BSL-2 laboratory.
5. β-Mercaptoethanol is to be used only in a chemical fume hood.

MATERIALS

Equipment and Consumables

- −80 °C freezer to store water samples
- −20 °C freezer to store detection reagents
- 4 °C refrigerator storage of samples and reagents
- P10, P100 or P200, and P1000 pipettes. It is strongly recommended to use different sets of pipettes for the extraction of RNA and RT-qPCR.
- Centrifuge (up to 12,000 x g) and rotor for 50 mL tubes
- Water bath
- Polypropylene 50 mL Oak Ridge Tubes (ThermoFisher™Cat. No. 3139-0030)
- Corning™ 50 mL tubes (Fisher Scientific Cat. No. 430290)
- Filter tips to fit all pipette sizes. Only filtered tips should be used for each step in the protocol.
- 150 mL Vacuum Filter/Storage Bottle System, 0.22 µm Pore 13.6cm² CA Membrane (Corning™ 430769)
- Alternatively, 0.22 µm syringe filters have been used (Filters: MilliporeSigma™ Cat. No. SLGP033RB; Syringes: Becton Dickinson™ Cat. No. 309654)
- Pasteur pipettes (Fisher Scientific™ Cat. No. 22-183632)
- 1.5mL microcentrifuge tubes (USA Scientific Cat. No. 1615-5510)
- 0.2 mL PCR tubes (Eppendorf™0030124847)
- Applied Biosystems™ MicroAmp™ Fast Optic 48-Well Reaction Plate (ThermoFisher Scientific Cat. No. 43-758-16).

Solutions/Reagents

- Polyethylene glycol 8000 (Fisher Scientific™ Cat. No. BP233-1)
- Sodium chloride (Fisher Scientific™Cat. No. BP358-10)
- TE buffer (Fisher Scientific Cat. No. BP2473500).
- TRIzol™ Reagent (Invitrogen™ Cat. No. 15596018)
- Chloroform (Fisher Scientific™ Cat. No. BP1145-1)
- Nuclease-free DEPC-treated water (Cat. No. AM9906) (10 x 50 mL)
- Ethanol, Absolute (200 Proof), Molecular Biology Grade (Fisher Scientific™ A4094)
- 2% Safranin Solution (Sigma-Aldrich Cat. No. 1092170500)
- Isopropanol (Fisher Scientific™ BP26181)
- Takara One Step PrimeScript III RT-PCR Kit (2x) (Takara™ RR064B)
- ROX Reference Dye (Invitrogen™ LS12223012)
- 2019-nCoV RUO N1 primers/probe (IDT) (Cat. No.10006713) (750 µL; 500 reactions)
- Custom BCoV qPCR assay (primers/probe)(IDT)
- Twist RNA (Cat. No. SKU# 102019)
- BCoV Standard gBlocks® Gene Fragment
- Calf-Guard® Bovine Rota Coronavirus vaccine (Zoetis, Cat. No. 540463)

SAFETY WARNINGS

- Wastewater is only to be worked with in a biosafety cabinet inside a BSL-2 laboratory.

Wastewater Fractioning and RNA Isolation

1d

1 Sample handling

The samples must be kept at 4°C at all times after collection. Keep wastewater sample containers closed throughout the entire procedure except where noted.

Wastewater preparation and fractioning is to be performed in a biosafety cabinet inside a BSL-2 laboratory.

2 Preparing wastewater sample

10m

1. Label 10 50 ml conical tubes MM/DD/YYYY, WW type, collection location (i.e., 01/12/2024, 24-hour composite, LANL TA-46)
2. Transfer 45 ml of wastewater to 10 50 ml conical tubes.
3. Add 5 ml 10X PBS pH 7.4 to each tube and mix thoroughly by inverting.
4. Store 5 of the 50 ml conical tubes at -80° C and proceed to the next steps with the remaining 5.

3 Fractioning wastewater

20m

1. Transfer 1 ml of wastewater from one of the 5 50 ml conical tubes and add it to a 5 ml PowerWater Bead Tube (**Fraction 1**).
2. Centrifuge the 5 50 ml conical tubes at 7000 x g for 10 minutes.
3. Carefully decant the supernatant into an appropriately sized (>500 ml) sterile beaker.
4. Combine the remaining liquid and pellets from each 50 ml conical tube into one of the 50 ml conical tubes.
5. Centrifuge the 50 ml conical tube at 7000 x g for 2 minutes.
6. Remove the supernatant from the tube.
7. Add 200 µl 1X PBS pH 7.4 to the pellet, and carefully transfer (by decanting and lightly scraping with a pipette tip) into a 5 ml PowerWater Bead Tube (**Fraction 2**).
8. Assemble a vacuum filter flask system with a 0.22 µm filter (Durapore® Membrane Filter, Millipore Sigma GVWP04700).
9. Pass the supernatant collected in step 4 through the filter for 15 minutes (filter may eventually become clogged).
10. Discard any remaining supernatant.
11. Disassemble the vacuum filter flask system.
12. Using sterile forceps, carefully roll the 0.22 µm filter and transfer it to a 5 ml PowerWater Bead Tube (**Fraction 3**).

Note: Fractions can be stored at 4° C for 72 hours.

4 RNA extraction and isolation

1h 30m

RNA extraction and isolation utilized the Qiagen RNeasy PowerWater Kit (Qiagen 14700-50-NF)

Before starting procedure:

1. Warm Solution PM1 at 55°C for 5-10 minutes prior to use.
2. Shake to mix Solution PM5 prior to use.
3. Prepare DNase I stock enzyme by adding 550 ul of RNase-Free water to the Dnase I (RNase-Free) lyophilized powder and mixing gently. Aliquot the DNase I stock enzyme in 50 ul proportions and store at -20°C for long term storage (but do not freeze/thaw more than 3 times). To prepare DNase I Solution, thaw and combine 5 ul of DNase I stock enzyme with 45 ul of DNase Digestion Solution per prep.

Sample lysis:

1. In a fume hood, add 990 ml of warm Solution PM1 and 10 ul of β-Mercaptoethanol (Millipore 444203) to the PowerWater Bead Tubes containing Fractions 1, 2, and 3.
2. Ensure that the caps of the PowerWater Bead Tubes are secure and place them horizontally in a vortex adapter with the caps pointing toward the center of the vortex adapter.
3. Vortex at maximum speed for 5 min.
4. Place PowerWater Bead Tubes in empty 50 ml conical tubes and centrifuge at 4000 x g for 1 min.
5. Remove PowerWater Bead Tubes from teh 50 ml conical tubes with forceps.
6. Transfer 1 ml of the supernatant by pipetting with a 1 ml pipette tip from down in the beads to clean 2 ml Collection Tubes.

RNA isolation:

1. Centrifuge 2 ml Collection Tubes at 13,000 x g for 1 min.
2. Avoiding the pellet, transfer the supernatant to clean 2 ml Collection Tubes.
3. Add 200 µl of Solution IRS and vortex briefly to mix. Incubate at 2-8°C for 5 min.
4. Centrifuge 2 ml Collection Tubes at 13,000 x g for 1 min.
5. Avoiding the pellet, transfer 650 µl the supernatant to clean 2 ml Collection Tubes.
6. Add 650 µl of Solution PM3.
7. Add 650 µl of Solution PM4.
8. Vortex briefly to mix.
9. Load 650 µl of supernatant onto an MB RNA Spin Column.
10. Centrifuge at 13,000 x g for 1 min. Discard flow through and repeat until all the supernatant has been loaded.
11. Add 650 µl of Solution PM5 and centrifuge at 13,000 x g for 1 min. Discard flow through.
12. Centrifuge again at 13,000 x g for 1 minute to dry membrane and place the MB RNA Spin Column into a clean 2 ml Collection Tube.
13. Add 50 µl of DNase I Solution to the center of the membrane and incubate at room temperature for 15 min.
14. Add 400 µl of Solution PM7 and centrifuge the column at 13,000 x g for 1 min.
15. Discard flow through and add 650 µl of Solution PM4, centrifuge at 13,000 x g for 1 min.
16. Discard flow through and centrifuge again at 13,000 x g for 2 min.
17. Place the MB RNA Spin Column into a clean 2 ml Collection Tube.
18. Add 100 µl of RNase-Free Water to the center of the white filter membrane.
19. Centrifuge at 13,000 x g for 1 min. Discard the MB RNA Spin Column.
20. Eluted RNA is ready for downstream applications.