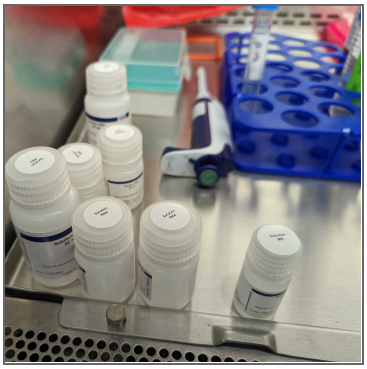

VERSION 2

AUG 30, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.q26g7p181gwz/v2

Protocol Citation: Michael Dan Siemon, Christelle Schang et al, Jessica Pardy, Richard Gibson, Dilan Joseph, Justin Donovan, christopher.degroot 2023. Qiagen RNEasy PowerMicrobiome RNA extraction kit. **protocols.io** https://dx.doi.org/10.17504/protocols.io.q26g7p181gwz/v2 Version created by Michael Dan Siemon

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Qiagen RNEasy PowerMicrobiome RNA extraction kit V.2

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ABSTRACT

The samples were processed using the Qiagen RNeasy PowerMicrobiome kit with the modifications described by Schang et al., 2021. As a substitute for vortexing described in the kit protocol, bead-beating was used. 100ul of phenol-chloroform-isoamyl alcohol was added to the bead beating tubes before the addition of the membranes. Bead-beating was conducted 4x for 30s at 4 m/s. After pelletizing the samples with centrifugation, the supernatant was processed through spin columns. Spin columns were incubated with DNase Digestion Solution for 15 minutes to isolate only RNA. The RNA was eluted from the spin columns by passing 50 uL of DEPC water through twice.

Protocol status: Working
This protocol was used for RNA extraction of wastewater samples for wastewater-based epidemiology at Western University in April 2023.

Created: Aug 30, 2023

Last Modified: Aug 30, 2023

PROTOCOL integer ID: 87168

IMAGE ATTRIBUTION

Michael Dan Siemon on behalf of ImPaKT Lab, University of Western Ontario

MATERIALS

Qiagen RNEasy PowerMicrobiome RNA extraction kit

Qiagen RNEasy PowerMicrobiome RNA

52m

1 Thoroughly mix wastewater sample then aliquot 40 mL into 50 mL Falcon tube. Centrifuge at 4500 x g for 20 min. Decant supernatant, assume 280 µl pellet. 20m

2 Add 100 µl phenol–chloroform–isoamyl alcohol to a PowerBead Bead Tube, Glass 0.1 mm. Place 0.25 g stool or biosolid sample into the Bead Tube.

3 Add 650 µl PM1 and 6.5 µl β-mercaptoethanol to the PowerBead Tube

4 As a substitute for vortexing described in the kit protocol, bead-beating was used. Bead-beating was conducted 4x for 30s at 4 m/s. 4m

5 After pelletizing the samples with centrifugation (13,000 x g for 1 min), mix the supernatant with 150 µl Inhibitor Removal Solution in a new collection tube and vortex briefly to mix. Incubate at 2–8°C for 5 min.

6 After pelletizing the samples with centrifugation (13,000 x g for 1 min), mix the supernatant with 650 µl each of Solution PM3 and Solution PM4. 1m

7 Process the solution through MB RNA Spin Column by centrifugation (13,000 x g for 1 min). Discard flow-through and repeat until all the solution has been processed. 3m

8 Shake solution PM5, add 650 µl to the Spin Column and centrifuge (13,000 x g for 1 min). 1m

9 Conduct a drying step by centrifuging at 13,000 x g for 1 min to remove residual wash. 1m

10 Incubate spin columns with DNase Digestion Solution for 15 minutes at room temperature to isolate only RNA. 15m

11 Add 400 µl Solution PM7 and centrifuge at 13,000 x g for 1 min. 1m

12 Discard flow-through. Add 650 µl Solution PM5. Centrifuge at 13,000 x g for 1 min. 1m

13 Discard flow-through. Add 650 µl Solution PM4. Centrifuge at 13,000 x g for 1 min. 1m

14 Conduct a drying step by centrifuging at 13,000 x g for 2 min. 2m

15 Elute RNA from the spin columns into 1.5 ml eppendorf tubes by passing 50 uL of DEPC water through twice. Centrifuge each elution 13,000 x g for 1 min. 2m