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Protocol status: Working
This protocol is used for wastwater RNA extraction for wastewater-based epidemiology at Western University

Qiagen QIAmp PowerFecal Pro extraction kit

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ABSTRACT

This protocol is used by Western University to process wastewater samples for wastewater-based epidemiology. The protocol is modified from the protocol described in the RNeasy PowerFecal Pro Kit Handbook provided by Qiagen with the PowerFecal Pro kit.

MATERIALS

This protocol requires the Qiagen QIAmp PowerFecal Pro extraction kit

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Sample Preparation

1 Thoroughly mix wastewater sample then aliquot 40 mL into 50 mL Falcon tube. Centrifuge at 4500 x g for 120 min. Decant supernatant, assume 280 µl pellet.

2 Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom. Add 100 µl of phenol–chloroform–isoamyl alcohol to the PowerBead Pro Tube.

3 Add 650 µl of Solution CD1 to the wastewater pellet and transfer solution to the PowerBead Pro Tube.

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

5 Centrifuge the PowerBead Pro Tube at 15,000 x g for 1 min.

6 Transfer the supernatant to a clean 2 ml Microcentrifuge Tube. Add 200 µl of Solution CD2 and vortex for 5 s.

7 Centrifuge at 15,000 x g for 1 min. Avoiding the pellet, transfer up to 650 µl of supernatant to a clean 2 ml Microcentrifuge Tube.

8 Add 650 µl of Solution EA. Vortex briefly.

9 Load 650 µl supernatant-EA mix into an MB RNA Spin Column and centrifuge at 15,000 x g for 1 min. Discard the flow-through and repeat until all of the solution has been passed through the Spin Column.

10 Add 650 µl Solution EA to the Spin Column and centrifuge at 15,000 x g for 1 min.

11 Mix 45 µl DNase Digestion Solution and 5 µl DNase I stock enzyme. Place the MB RNA Spin Column into a clean 2 ml Collection Tube and add the DNase Digestion Solution to the center of the filter. Incubate at room temperature for 15 minutes.

12 Add 650 µl Solution EA to the Spin Column and centrifuge at 15,000 x g for 1 min.

13 Discard the flow-through. Add 500 µl Solution C5. Centrifuge at 15,000 x g for 1 min.

14 Replace the collection tube and dry the Spin Column by centrifuging at 20,000 x g for 1 min.

15 Elute the RNA by placing the spin column in a 1.5 ml collection tube. Add 100 µl of RNase-free water and centrifuge at 15,000 x g for 1 min.