

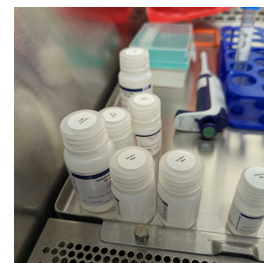
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Version 2

Qaigen RNEasy RNA extration protocol V.2

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External link: <https://www.qiagen.com/us/resources/download.aspx?id=c80685c0-4103-49ea-aa72-8989420e3018&lang=en>

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Protocol status: Working

This protocol was used for RNA extraction of wastewater samples for wastewater-based epidemiology at Western University.

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Abstract

This protocol was employed by Western University for RNA extraction of wastewater samples for wastewater-based epimelogy in London, Ontario, Canada and surrounding area. The protocol was adapted from QIAamp® Viral RNA

Mini Handbook for use with the Qiagen RNeasy extraction kit.

Materials

This protocol requires QIAamp RNeasy viral minikit - Qiagen



Protocol modified from QIAamp® Viral RNA Mini Handbook

- 1 Thoroughly mix wastewater sample then aliquot 40 mL into 50 mL Falcon tube. Centrifuge at 12000 RPM for 90 min. Decant supernatant, assume 280 µl pellet.
- 2 Pipet 1120 µl prepared Buffer AVL into Falcon tube with sample
- 3 Pulse-vortex samples for 15s every 2 minutes for 8 minutes
- 4 Add 1120ul ethanol then centrifuge at 4000RPM for 5 minutes
- 5 Process supernatant through QiAmp Mini column by adding 750 µl and centrifuging at 8000 rpm for 1 min. Remove flow-through and repeat until all supernatant has been processed
- 6 Add 500 µl Buffer AW1 to QiAmp Mini column and centrifuge at 6000 x g (8000 rpm) for 1 min. Replace the collection tube with a clean 2 ml collection tube
- 7 Add 500 µl Buffer AW2 to QiAmp Mini column and centrifuge at 6000 x g (8000 rpm) for 1 min. Replace the collection tube with a clean 2 ml collection tube
- 8 Dry membrane by centrifuging at 13000 rpm for 3 min
- 9 Place the QIAamp Mini column in a clean 1.5 ml eppendorf tube. Add 60 µl Buffer AVE equilibrated to room temperature.
- 10 Centrifuge at 6000 x g (8000 rpm) for 1 min

Protocol references

Adapted from:

QIAamp Viral RNA Mini Handbook—QIAGEN. (2020.). Retrieved August 24, 2023, from
<https://www.qiagen.com/us/resources/resourcedetail?id=c80685c0-4103-49ea-aa72-8989420e3018&lang=en>