

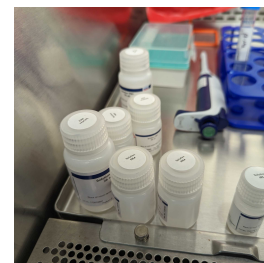
Aug 30, 2023

Version 1

Qaigen RNEasy RNA extration protocol V.1

DOI

<https://dx.doi.org/10.17504/protocols.io.dm6gp337dvzp/v1>



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

Protocol Citation: Jessica Pardy, Michael Dan Siemon, Dilan Joseph, Justin Donovan, christopher.degroot , Richard Gibson 2023. Qaigen RNEasy RNA extration protocol. protocols.io <https://dx.doi.org/10.17504/protocols.io.dm6gp337dvzp/v1>

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Protocol status: Working

This protocol was used by Western University for RNA extraction of wastewater samples for wastewater-based epidemiology. It was used from 2020 to April 2023.

Created: August 25, 2023

Last Modified: August 30, 2023

Protocol Integer ID: 86937

Keywords: qiagen rneasy rna extration protocol, qiagen rneasy rna extraction protocol this protocol, qiagen rneasy extraction kit, rna extraction, western university for rna extraction, viral rna, rna, wastewater samples for wastewater, wastewater sample, wastewater, qiagen

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

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>