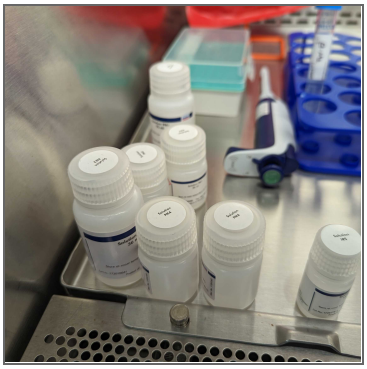

VERSION 2

AUG 31, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.dm6gp337dvzp/v2

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, Richard Gibson, christopher.degroot, Amanda Hamilton 2023. Qaigen RNEasy RNA extration protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.dm6gp337dvzp/v2
Version created by Michael Dan Siemon

Qaigen RNEasy RNA extration protocol V.2

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

Created: Aug 31, 2023

Last Modified: Aug 31, 2023

PROTOCOL integer ID: 87217

Keywords: wastewater-based epidemiology, Qiagen, RNA extraction, ImPaKT

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