Promega

Product Application

Purification of Viral RNA from Wastewater Concentrates

Purify viral RNA from wastewater concentrates using the Maxwell® RSC Instrument and Maxwell® RSC PureFood GMO and Authentication Kit.

Kit: Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)

Analyses: RT-qPCR

Sample Type(s): Wastewater concentrates

Input: Up to 200µl of concentrate

Materials Required:

- Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Heat block or waterbath set to 56°C
- 1.5ml tubes

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM473, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Protocol:

Wastewater concentrates may be prepared by any method such as viral precipitation/flocculation or filtration. An example protocol for preparing wastewater viral concentrates by precipitation with NaCl and PEG 8000 is given below. If wastewater concentrates were prepared by an alternative method, proceed directly to Purification of Viral RNA from Wastewater Concentrates, Step 1 below.

Preparation of the wastewater concentrates by PEG 8000/NaCl precipitation[1] (optional).

a. Centrifuge wastewater at 4,000 x *g* for 30 minutes to pellet particulates.
b. By pipetting, carefully transfer 40ml of the supernatant to a clean vessel. Avoid disturbing the pellet at the bottom of the tube.
c. Per 40ml of wastewater, add 4g of PEG 8000 and 0.9g of NaCl. Gently mix until completely dissolved.
d. Centrifuge sample at 11,400 x *g* for 2 hours.
   Note: It is important to mark the side of the tube on which a pellet would be expected to form. The pellet formed may not be visible.
e. Remove liquid from the tube by carefully pipetting with a serological pipette from the side of the tube opposite of where a pellet should have formed.
f. Resuspend the pellet in the residual liquid in the tube by vortexing.

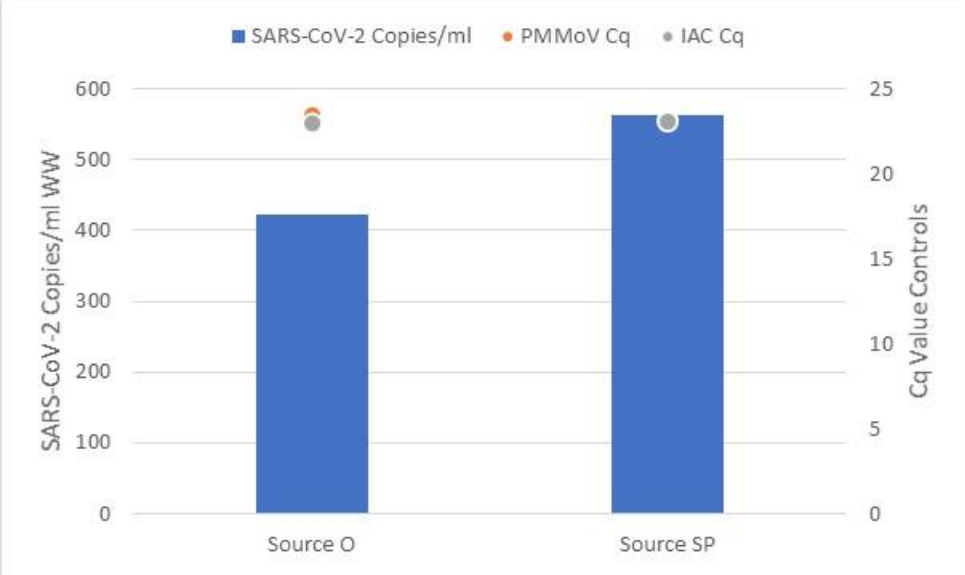
Purification of viral RNA from wastewater concentrates.

1. Combine ≤200µl of wastewater concentrate with 200µl of CTAB and 40µl of Proteinase K. Vortex for 10 seconds.
2. Incubate sample at 56°C for 10 minutes.

3. Add entire sample and 300µl of Lysis Buffer to well #1 of the Maxwell® RSC cartridge. Place a plunger in well #8 of the Maxwell® cartridge, and add 50µl of Elution Buffer to the bottom of the provided Elution Tube. Place the Elution Tube in the deck tray with the cap open.
4. Process samples with the PureFood GMO and Authentication protocol on the Maxwell® RSC Instrument.

Results:

SARS-CoV-2 RNA was successfully detected in nucleic acid purified from wastewater concentrates prepared by precipitation with NaCl and PEG 8000.



SARS-CoV-2 detected in nucleic acid purified from wastewater. Wastewater samples from two municipalities (Source O, Source SP) were collected in December 2020, stored at 4°C, and processed within 48 hours of collection. 40ml of each wastewater sample was processed for nucleic acid purification as described above, using wastewater concentrates prepared by precipitation with NaCl and PEG 8000. Following purification, 2µl of each eluate was assayed for SARS-CoV-2 RNA with the SARS-CoV-2 RT-qPCR Kit for Wastewater, a multiplex assay that amplifies a nucleocapsid SARS-CoV-2 target (N2, blue bars) and, as controls, the Pepper Mild Mottle Virus (PMMoV, orange points)—the most abundant viral RNA found in human feces, and an RNA internal amplification control target (IAC, gray points) used to assess amplification inhibition. Viral copies in the eluates were determined relative to a quantitative control standard curve and converted to copies/ml input of wastewater. Average (n =2) is shown.

Reference:

1. Medema, G., et al., (2020) Presence of SARS-Coronavirus-2 RNA in Sewage and Correlation with Reported COVID-19 Prevalence in the Early Stage of the Epidemic in The Netherlands. *Environ. Sci Technol. Lett.* 2020; 7(7): 511-516.