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Extraction of Total Nucleic Acid from Wastewater Using the Promega Wizard Enviro Total Nucleic Acid Kit

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Wastewater based epidemiology has proven to be a useful tool in the COVID-19 pandemic, allowing prevalence and variant and sub-lineage surveillance on a watershed population level in a non-intrusive manner. Essential to this method is the efficient extraction of viral genomic material from wastewater. The process of detecting SARS Co-V-2 genetic signatures in wastewater samples involves collection of water, either as a grab sample or as a 24-hour composite sample, followed by sample concentration. Target genetic material may be present at a low concentration in water samples, making sample concentration a prerequisite for sensitive detection. Concentration of microbial matter can be performed using a variety of methods, such as charged membrane filtration, centrifugal ultrafiltration and flocculation/precipitation using skim milk or polyethylene glycol (PEG)/NaCl. Most of the concentration methods were originally developed to concentrate live matter with the objective of culturing for detection of intact particles, though they have also been used for PCR-based detection. These methods have proven to be inconsistent, labor intensive and time consuming.

More and more SC2-wastewater specific methods have recently been developed. We have assessed the Promega Wizard Enviro Total Nucleic Acid kit for extraction of RNA for downstream SC2 detection and sequencing. Briefly, the method directly captures and concentrates total nucleic acids (TNA) from a large volume of water using PureYield™ columns. The method uses a short protocol that minimizes the need for specialized laboratory equipment. In a first step total nucleic acid from a large volume sample (e.g., 40ml of wastewater) is captured on a PureYield™ Binding Column and then eluted in 1ml. In a second step, the material is further purified and concentrated using the PureYield™ Minicolumn. This method achieves consistent recovery rates and significant reduction in PCR inhibitors.

The total nucleic acid extracted using this kit can be analyzed for SARS-CoV-2 targets using a SARS-CoV-2 RT-qPCR
kit for wastewater. Please visit the Promega website for more information on these products:
https://www.promega.com/applications/infectious-diseases/covid19-wastewater-sars-cov-2-detection/. Additionally, the extracted RNA can be utilized for SC2 variant sequencing: Enhanced QIAseq DIRECT SARS-CoV-2 Kit for Illumina MiSeq (protocols.io), or Modified NEBNext® VarSkip Short SARS-CoV-2 Library Prep Kit for Illumina Platforms - adapted for wastewater samples (protocols.io).

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Wizard® Enviro Total Nucleic Acid Kit, Promega cat# A2991
Contains sufficient materials and reagents for 25 samples. Includes:
• 1 × 13ml Protease Solution
• 1 × 320ml Binding Buffer 1 (BBD)
• 1 × 30ml Binding Buffer 2 (BBE)
• 1 × 85.3ml Column Wash 1(CWE)
• 1 × 206ml Column Wash 2 (RWA)
• 1 × 150ml Nuclease-Free Water
• 5 × 5 each PureYield™ Binding Columns
• 1 × 25 each PureYield™ Minicolumns
• 1 × 25 each PureYield™ Collection Tubes
• 1 × 50 each Elution Tubes
• 1 × 25 each Reservoir Extension Funnel
Storage Conditions: Store all components at +15°C to +30°C.

Materials to Be Supplied by the User
• isopropanol
• ethanol, 95%
• tabletop centrifuge (capable of 3,000 × g)
• swinging bucket rotor (that accommodates 50ml tubes)
• 50ml disposable plastic screw-cap tubes (e.g., Corning® or Falcon® brand)
• 1.5ml microcentrifuge tubes
• heat block (capable of reaching 60°C)
• high-speed microcentrifuge (for tubes, capable of at least 10,000rpm)
• vacuum manifold (e.g., Vac-Man® Laboratory Vacuum Manifold, Cat.# A7231)
• Eluator™ Vacuum Elution Device (Cat.# A1071)
• vacuum pump, single- or double-stage, producing pressure of approximately 650mm Hg (25.6 inches Hg,
12.57psi, 86.7kPa).

Prepare the following solutions prior to beginning nucleic acid extraction in Section 4:

Column Wash 1 (CWE): Add **57 mL** of isopropanol to the Column Wash 1 (CWE) bottle and mark on the bottle “plus
isopropanol”. This reagent is stable at **15 °C** to **30 °C** when tightly capped.

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Column Wash 2 (RWA): Add **350 mL** of **95 % (v/v)** ethanol the Column Wash 2 (RWA) bottle and mark the bottle “plus ethanol”. This reagent is stable at **15 °C** to **30 °C** when tightly capped.

Turn on water bath to **60 °C**

Capture and Concentration

1 Dispense **40 mL** of wastewater or pasteurized wastewater into a

50 mL conical screw-cap tube
Plastic consumables

Grenier Bio-One **5622-7261**

Any brand or size of plastic tube with a tight-fitting screw cap will work fine.

Pasteurizing wastewater is optional. To pasteurize wastewater, incubate at **60 °C** for **01:00:00** Please follow your institution’s biosafety guidelines.

2 Add **500 µL** of Protease Solution. Mix well by inversion and incubate for **00:30:00** at **Room temperature** . 30m

3 **3000 x g, Room temperature, 00:10:00** . 10m

This step is designed to remove solids, to avoid clogging the PureYield™ Binding Column.

4 Carefully decant **20 mL** of the supernatant into each of two clean

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50 mL conical screw-cap tube
Plastic consumables

Grenier Bio-One **5622-7261**

Any brand or size of plastic tube with a tight-fitting screw cap will work fine.

Discard the 50ml conical tube containing the pellet into an appropriate biohazard waste container.

Sometimes, the solids will produce a loose pellet. In this case, let the solids settle again before decanting or use a serological pipet to avoid transferring solids to the binding column.

If you wish to process the pelleted solids to collect additional total nucleic acid, see Section 5.A. of the Wizard® Enviro Total Nucleic Acid Kit Technical Manual.

5 To each tube containing **20 mL** of the clarified supernatant, add **6 mL** of Binding Buffer 1 (BBD) followed by **500 µL** of Binding Buffer 2 (BBE).

6 Mix well by inverting the tube gently 10 times, or until thoroughly mixed.

7 Add **24 mL** of isopropanol to each tube.

8 Mix well by inverting the tube gently 10 times, or until thoroughly mixed.

9 Setup the vacuum manifold as follows (see Figure 1): remove vacuum port cap, attach a Reservoir Extension Funnel to the PureYield™ Binding Column, then connect the column to the

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vacuum manifold by pressing the nozzle gently into the vacuum port.

Figure 1. The Reservoir Extension Funnel and PureYield™ Binding Column attached to a Vac-Man® Vacuum Manifold port. See Quick Start Guide for Assembly of a Vacuum Apparatus with the Welch Vacuum Pump
Technical Bulletin #TB355 for setup details.

Using the Reservoir Extension Funnel allows up to **100 mL** of sample mixture to be added to the PureYield™ Binding Column at one time.

Due to the size of the assembled binding column and funnel, we suggest processing 6 to

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8 samples at a time per vacuum manifold.

10 Pour the mixture from each tube from Step 8 into the Reservoir Extension Funnel on the PureYield™ Binding
Column (combine both tubes of the same sample if applicable), turn on the pump and apply vacuum to capture
TNA on the column.

Periodically, empty the liquid waste collected in the blue Vac-Man® Laboratory Vacuum Manifold (Cat.# A7231). Dispose of the alcohol-containing waste following your institutional policies.

11 Add **5 mL** of Column Wash 1 (CWE) and apply a vacuum to pull the liquid through the PureYield™ Binding
Column.

Ensure that **57 mL** of isopropanol has been added to the Column Wash 1 (CWE) bottle prior to use as described in the "Before Start" section.

12 Add **20 mL** of Column Wash 2 (RWA) and apply a vacuum to pull the liquid through the PureYield™ Binding
Column. Continue to draw a vacuum for an additional **00:00:30** after all visible liquid has passed through the
membrane. 30s

Ensure that **350 mL** of **95 % (v/v)** ethanol has been added to Column Wash 2 (RWA) bottle
prior to use as described in the "Before Start" section.

13 Release the vacuum and remove the column from the vacuum manifold. Preheat **1.2 mL** of Nuclease-Free Water,
per sample, to **60 °C** for **00:05:00** . 5m

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14 Assemble the elution device by placing a 1.5ml microcentrifuge tube into the base of the Eluator™ Vacuum
Elution Device (Cat.# A1071) and securing the tube cap in the open position, as shown (Figure 2).
Insert the PureYield™ Binding Column into the top of the Eluator Device, making sure the column is fully seated
on the collar as shown in Figure 3.

Figure 2. A 1.5ml microcentrifuge tube is placed in the base of the Eluator™ Vacuum Elution Device with the tube cap locked as shown.

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Figure 3. The final Eluator™ Vacuum Elution Device assembly, including the binding column, ready for use on a vacuum manifold.

15 Place the Eluator™ Device assembly onto a vacuum manifold (Figure 3). Add **500 µL** of [1m] preheated (**60 °C**) Nuclease-Free Water to the PureYield™ Binding Column. Apply maximum vacuum for **00:01:00** or until all liquid has passed through the column. Repeat the process by adding another **500 µL** of preheated Nuclease-Free Water to the PureYield™ Binding Column to elute a total of **1 mL** of TNA solution.

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TNA Extraction and Clean-Up 11m 30s

16 Add **400 µL** of Binding Buffer 1(BBD) and **100 µL** of Binding Buffer 2 (BBE) to **1 mL** of liquid eluted in
Step 15.

17 Mix well by inverting the tube gently 10 times, or until thoroughly mixed. Divide the contents into two

DNA LoBind Tube 1.5 mL
Microcentrifuge tube
Eppendorf **022431021**

containing **750 µL** each.

18 **00:01:00** Add **750 µL** of isopropanol to each tube and mix well by inverting the tube gently 10 times, or until thoroughly mixed. 1m

19 Place the PureYield™ Minicolumn into a PureYield™ Collection Tube. Pass the entire volume of the mixture
through the column, **750 µL** at a time (a total of four times), using a microcentrifuge at **10.000 rpm, Room temperature, 00:01:00** . 1m

20 Add **300 µL** of Column Wash 1 (CWE) and pull through the PureYield™ Minicolumn by centrifugation **10000 rpm, Room temperature, 00:01:00** . Discard the flow-through. 1m

21 Add **500 µL** of Column Wash 2 (RWA) and pull through the PureYield™ Minicolumn by centrifugation **10000 rpm, Room temperature, 00:01:00** . Repeat this wash one time. Discard the flowthrough. 1m

22 Centrifuge **10000 rpm, Room temperature, 00:00:30** to remove any residual wash solution. 30s

23 Preheat **65 µL** of Nuclease-Free Water per sample to **60 °C** for **00:05:00** . 5m

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24 Transfer the PureYield™ Minicolumn to a new 1m

DNA LoBind tubes, 1.5 mL
Tubes
Eppendorf **022431021**
1.5 mL

and add **30 µL** of preheated (**60 °C**) Nuclease-Free Water to the column. Let the water soak into the column filter for approximately **00:01:00** .

25 Centrifuge **10000 rpm, Room temperature, 00:01:00** to elute. Repeat elution with another **30 µL** of preheated Nuclease-Free Water, for a total of **60 µL** . 1m

26 Store sample at or below **-20 °C** until further analysis. TNA purified using this method can be directly used for
RT-qPCR, for example as input into the following protocol:
RTqPCR of SARS-CoV-2 N1 Target on ABI 7500 Fast Using Promega GoTaq Enviro Wastewater SARS-CoV-2 System V1 (protocols.io)

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